

Annals of *Research* in *Oncology*

www.annals-research-oncology.com

EDITORIAL

CANCER RESEARCH
AND CARE IN THE
FUTURE POST-
COVID-19 ERA

OPINION PAPER

CLINICAL RESEARCH
DURING COVID-19
SPREAD: MANAGING
AN EMERGENCY
WITHIN A PANDEMIC
OUTBREAK

OPINION PAPER

RESEARCHERS'
PYRAMID. A NEW
OPPORTUNITY
FOR ITALIAN
ONCOLOGY RESEARCH
INFRASTRUCTURE?

RESEARCH ARTICLE

EGFR+ GLIOBLASTOMA
STEM CELLS
TARGETING BY
CD16158V-CHIMERIC
RECEPTOR T CELLS
AND CETUXIMAB

EDITORS IN CHIEF

A. Giordano
C. Pinto

EXECUTIVE EDITOR

F. Pentimalli

SECTION EDITORS

Cancer Epidemiology and Prevention	Viruses and Cancer
D. Serraino	A. Petruzzello
Cancer Biomarkers	Nutrition and Cancer
M. Barbareschi	R. Caccialanza
M. Barberis	P. Pedrazzoli
Cancer Genetics, Epigenetics and non coding RNAs	Palliative Care
R. Benedetti	A. Caraceni
N. Del Gaudio	Cancer and Society
Cancer Signalling and Molecular Mechanisms	Breast Cancer
A. Morrione	F. Montemurro
Cancer Metabolism	Thoracic Cancer
C. Mauro	M. Di Maio
M. Vanoni	L. Mutti
Cancer Inflammation, Microenvironment and Metastasis	Head and Neck Cancer
S. Mani	M. Benasso
Cancer Immunology and Immunotherapy	Endocrine System Cancer
A. Grimaldi	P. Scalia
Cancer Therapy and Precision Medicine	Gastrointestinal Cancer
F. Graziano	F. De Vita
Cancer Pharmacology	Genitourinary Cancer
R. Danesi	O. Caffo
G. Toffoli	G. Procopio
Cancer Screening	Gynaecological Cancer
P. Giorgi Rossi	S. Pignata
Cancer Drug Discovery and Repurposing	Neurooncology
T. Tuccinardi	A. Brandes
Cancer Supporting Care	Sarcoma
D. Corsi	A. Comandone
Cancer Imaging and Radiotherapy	G. Grignani
E. Russi	Melanoma and Skin Cancer
Cancer Clinical Trials	M. Mandalà
G. Daniele	G. Palmieri
	Rare Cancers
	N. Fazio
	B. Vincenzi
	Consultant For Biostatistics
	G. Baglio

EDITORIAL BOARD

L. Alfano (Italy)	R. Franco (Italy)
L. Altucci (Italy)	P. Indovina (Philadelphia)
M. Barbarino (Philadelphia)	R. Lucchini (Italy)
A. Feliciello (Italy)	D. Ruggiero (San Francisco)
E. Franceschi (Italy)	

Editors in Chief and Executive Editor

Antonio Giordano
Carmine Pinto
Francesca Pentimalli

Chief Business & Content Officer

Ludovico Baldessin

Editorial Coordinator

Barbara Moret

Publishing Editor

Elisa Grignani
e.grignani@lswr.it
Ph. 0039 (02) 8929 3925

Sales

Stefano Busconi
dircom@lswr.it
Ph. 0039 (0)2-88184.404

EDRA SpA

Via G. Spadolini, 7
20141 Milano - Italy
Tel. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.edizioniedra.it

"Annals of Research in Oncology" registered at Tribunale di Milano
n. 63 on 24.06.2020

© 2021 Annals of Research in Oncology - ARO.
Published by EDRA SpA. All rights reserved.

To read our Privacy Policy please visit www.edraspa.it/privacy

Table of contents

Cancer research and care in the future post-covid-19 era	4
C. Pinto, A. Giordano, F. Pentimalli	
Clinical research during COVID-19 spread: managing an emergency within a pandemic outbreak	7
C. Cagnazzo, S. Testoni, R. Cenna, S. Stabile, E. Tirtei, N. Bertorello, F. Fagioli	
Researchers' Pyramid. A new opportunity for Italian oncology research infrastructure?	13
S. Testoni, R. Cenna, A.S.E. Guarrera, M. Monti, S. Stabile, I. Federici, C. Taverniti, S. Pirondi, A. Ferrari, C. Cagnazzo	
EGFR⁺ glioblastoma stem cells targeting by CD16^{158V}-chimeric receptor T cells and cetuximab	24
C. Cenciarelli, S. Caratelli, R. Arriga, G. Lanzilli, A. Ottaviani, T. Sconocchia, G. C. Spagnoli, A. Venditti, G. Iezzi, M. Roselli, D. Lauro, H. E. Marei, G. Sconocchia	
The impact of Covid-19 on new cancer diagnoses	36
L. Mangone, P. Giorgi Rossi, I. Bisceglia, R. Grilli, C. Pinto	
Prevention of oxaliplatin-induced peripheral neuropathy in colorectal cancer: The PLANET trial experience	43
S. Delfanti, R. Tancredi, F. Serra, T. Monaco, C. Gandini, A. Ferrari, C. Tinelli, S. Brugnattelli	
The resilience of oncologists during the early time of the covid-19 pandemic	55
A. Lasagna, S. Secondino, F. Agustoni, T. Monaco, I. Imarisio, A. Pagani, G. Rizzo, R. J. Tancredi, E. Pozzi, E. Ferraris, S. Chiellino, C. Gandini, A. Ferrari, S. G. Brugnattelli, P. Pedrazzoli	
Impact of different dosing strategies of nivolumab in patients with solid tumors: Italian single center analysis	61
T. Gamba, A. Caglio, F. Sacchi, C. Paratore, G. Fazzina, V. Tagini, M. Bellerio, S. Masucci, F. Vignani, G. Lacidogna, C. Zichi, G. Aimar, D. Marino, E. Sperti, A. Gasco, M. Di Maio	
Safety matters: the troubled and finally successful story of dihydropyrimidine dehydrogenase pharmacogenetic test in cancer patients	71
S. Crucitta, F. Sciandr, A. Cerbioni, F. Cucchiara, R. Arici, C. Rafaniello, A. Capuano, R. van Schaik, R. Danesi, M. Del Re	
Combination immunotherapy in metastatic renal cell carcinoma	85
P. Sepe, A. Ottini, E. Verzoni, V. Guadalupi, M. Claps, G. Procopio	

EDITORIAL

CANCER RESEARCH AND CARE IN THE FUTURE POST-COVID-19 ERA

A. Giordano ^{1,2}, C. Pinto ³, F. Pentimalli ⁴

¹ Department of Medical Biotechnologies, University of Siena, Siena, Italy

² Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, PA, USA

³ Oncologia Medica, Clinical Cancer Center, AUSL-IRCCS, Reggio Emilia, Italy

⁴ Cell Biology and Biotherapy Unit, Istituto Nazionale Tumori-IRCCS-Fondazione G. Pascale, Naples, Italy

CORRESPONDING AUTHOR:

Francesca Pentimalli
Cell Biology and Biotherapy Unit
Istituto Nazionale Tumori
IRCCS-Fondazione G. Pascale
via Mariano Semmola 52
80131 Naples, Italy
E-mail: f.pentimalli@istitutotumori.na.it

Doi: 10.48286/aor.2021.01

The community of cancer researchers has made tremendous progress against cancer in the past decades. The number of persons who survived cancer has been steadily increasing (1) while the cancer death rate has been continuously declining from its peak in 1991 to a reduction of 31% in 2018 in the US, meaning that 3.2 million fewer cancer deaths occurred than we could have witnessed if those peak rates had persisted (2). Such positive trends and figures are due to our overall improved ability to prevent, detect and also treat cancer, being often able to make the disease chronic rather than lethal, although this progress has not been uniform for all cancer types, subtypes or stages (2). Despite current efforts, however, cancer is mostly a disease of ageing and the number of persons, aged 60 years or more, is projected to double by 2050 reaching 1.5 billion worldwide (3). There is therefore raising concern about a growing population burden of cancer; moreover, the current COVID-19 pandemic has significantly impacted on

the continuum of cancer care (4) delaying cancer screening, diagnosis, and treatment, likely worsening previous projections.

Unfortunately, the expected high burden of cancer will exact a high toll not only in terms of lives but also from an economic point of view: cancer will still be a major public health challenge in the next decades bringing National Health Systems at risk of collapse. In this scenario and with the current high level of knowledge and technologies available, are 'we' - as citizens, researchers, doctors, politicians - doing all we possibly can to tackle cancer? Probably not. The rush to identify ways to detect and treat COVID-19 has thought us that when governments, pharma, public and private institutes invest hugely in research, when all the scientific community, publishers, regulatory agencies are committed towards the same goal, progress can take place at an unprecedented pace. It is time to rethink our strategies to better direct our common efforts to defeat cancer. With this objective in mind, we are launching *An-*

nals of Research in Oncology, a new e-journal aimed at promoting a multidisciplinary integrated approach against cancer serving as a platform for the publication of cutting edge research, spanning the broad areas of basic, translational and clinical oncology. The journal stems as the need of a community of scientists, equally engaged in the war against cancer, to build bridges across the many different disciplines that study cancer in each possible aspect and to promote collaboration, debate and development of innovative solutions.

On these bases, this first issue of *Annals of Research in Oncology* brings together a set of articles focused on different themes. The article by Mangone and colleagues reports the impact of the National lockdown owed to COVID-19 on cancer diagnosis in Reggio Emilia, an Italian province in the Northern area that was heavily hit by the disease (5). The authors analyzed the incidence of all cancers and of cancer of the major sites registered pre-, during and post-lockdown compared to same months in the previous year. Consistently with expectations, they found a decrease in cancer diagnoses, in particular for those cancer types for which screening programmes are conducted (breast and colorectal) and in the older people. The authors suggest that diagnostic programmes need to be resumed at the earliest to limit the impact of diagnostic delay on patients prognosis (5). Lasagna and colleagues instead propose a simple double-step triage strategy that functioned in maintaining cancer patient and health care worker safety during COVID-19 emergency (6). Still on the impact of COVID-19, Cagnazzo and coauthors (7) analyze some major criticalities concerning cancer research and management during the pandemic, including the effect on the ongoing clinical trials and on how the emergency showed the crucial role of expert healthcare professionals, proposing new strategies for the future. Stressing the importance of the need of qualified personnel, Testoni *et al.* (8) describe a new path for the possible stabilization of researchers who have at least three years of experience in Institutions of the National Health System, which was recently introduced in Italy. Italy neglected for too many years research investments and, despite spending on training at considerable costs, many scientists are forced to leave the country while those who stay are left with very poor prospects. COVID-19 once again showed all the insanity of such unwise policies: the governors had to rush to recruit health personnel during the pandemic, even including those who had not com-

pleted the full specialization programme.

The recent advent of immunotherapy, as a new fundamental pillar of cancer therapy, has challenged the health systems because of the high cost of immunotherapy drugs. The article by Di Maio and colleagues analyzes the cost-effectiveness of different dosing schemes of nivolumab in the real world suggesting a strategy that could minimize costs without losing efficacy (9).

Cenciarelli and coauthors present a new possible strategy that could possibly counteract some types of glioblastoma recurrence. In particular, the authors engineered T cells with Fcγ-chimeric receptors, which are able to elicit antibody-dependent cellular cytotoxicity, and challenged glioblastoma-derived EGFR+ cancer stem cells in combination with monoclonal antibodies against EGFR. Interestingly, this approach was able to induce cell death in the target cancer cells while T cells generated a fully competent immune response including INFγ and TNFα expression upon recognition of target cells (10).

Delfanti and colleagues report the results of the PLANET trial, a monocentric, prospective, randomized, placebo-controlled, double blind, phase II clinical study designed to assess whether the association of vitamin E and super oxide dismutase could prevent oxaliplatin-induced peripheral neuropathy in colorectal cancer patients. Such neuropathy is a common side effect in patients receiving this chemotherapy regimen. Although the treatment was well tolerated however it was not effective in significantly reducing the toxicity making the authors hypothesize that further approaches should be combined to counteract the multifactorial origins of the neuropathy (11).

Finally, this first issue of *Annals of Research in Oncology* hosts two review articles focused on timely topics: Sepe *et al.* (12) describe the current use of combination immunotherapy approaches to treat and tackling resistance of metastatic renal cell carcinoma, whereas Crucitta *et al.* review fluoropyrimidines metabolism and discuss how gene variants that impair dihydropyrimidine dehydrogenase activity can cause severe toxicities in patients treated with fluoropyrimidine-based chemotherapy, further supporting the recent recommendations for the implementation of pharmacogenomic testing in these patients (13).

We hope that our readers will enjoy this issue and join our community to rethink the way we fight cancer, being more engaging, better focusing our global efforts, better harnessing our technology tools.

REFERENCES

1. American Association for Cancer Research. AACR cancer progress report 2019. Available at: <http://www.cancerprogressreport.org>.
2. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin* 2021;71(1):7-33.
3. United Nations. World Population Ageing 2019: Highlights 2019. Available at: <https://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeing2019-Highlights.pdf>.
4. Hans Henri P. Kluge. Statement-Catastrophic impact of COVID-19 on cancer care, World Health Organization, Regional Office for Europe 2021. Available at: <https://www.euro.who.int/en/media-centre/sections/statements/2021/statement-catastrophic-impact-of-covid-19-on-cancer-care>.
5. Mangone L, Giorgi Rossi P, Bisceglia I, Grilli R, Pinto C. The impact of covid-19 on new cancer diagnoses. *Ann Res Oncol* 2021;(1).
6. Lasagna A, Secondino S, Agustoni F, et al. Cancer care at the time of covid-19: practical approach to the management of patients during the early time of the pandemic in a large Italian community hospital. *Ann Res Oncol* 2021;1.
7. Cagnazzo C, Testoni S, Cenna R, et al. Clinical research during COVID-19 spread: managing an emergency within a pandemic outbreak. *Ann Res Oncol* 2021;1.
8. Testoni S, Cenna R, Guarrera ASE, et al. Researchers' Pyramid. A new opportunity for Italian oncology research infrastructure. *Ann Res Oncol* 2021;1.
9. Gamba T, Caglio A, Sacchi F, et al. Impact of different dosing strategies of nivolumab in patients with solid tumors: Italian single center analysis. *Ann Res Oncol* 2021;1.
10. Cenciarelli C, Caratelli S, Arriga R, et al. EGFR+ glioblastoma stem cells targeting by cd16158v-chimeric receptor t cells and cetuximab. *Ann Res Oncol* 2021;1.
11. Delfanti S, Tancredi R, Serra F, et al. Prevention of oxaliplatin-induced peripheral neuropathy in colorectal cancer: the planet trial experience. *Ann Res Oncol* 2021;1.
12. Sepe P, Ottinia A, Verzonja E, Guadalupia V, Clapsa M, Procopio G. Combination Immunotherapy in Metastatic Renal Cell Carcinoma. *Ann Res Oncol* 2021;1.
13. Crucitta S, Sciandra F, Cerbioni A, et al. Safety matters: the troubled and finally successful story of DPD testing in cancer patients. *Ann Res Oncol* 2021;1.

OPINION PAPER

CLINICAL RESEARCH DURING COVID-19 SPREAD: MANAGING AN EMERGENCY WITHIN A PANDEMIC OUTBREAK

C. Cagnazzo ^{1,2}, S. Testoni ³, R. Cenna ², S. Stabile ⁴, E. Tirtei ^{1,2}, N. Bertorello ²,
F. Fagioli ^{1,2}

¹ Department of Public Health and Pediatrics, University of Turin, Turin, Italy

² Paediatric Haematology-Oncology Department, Regina Margherita Children's Hospital, A.O.U. Città della Salute di Torino, Turin, Italy

³ IRCCS Istituto Scientifico Romagnolo per lo Studio dei Tumori (IRST) Dino Amadori, Meldola, Italy

⁴ Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milano, Italy

CORRESPONDING AUTHOR:

Celeste Cagnazzo
Department of Public Health and Pediatrics University of Turin
S.C. Oncoematologia Pediatrica
AOU Città della Salute e della Scienza
Presidio Ospedaliero Infantile Regina Margherita
piazza Polonia 94
10126 Turin, Italy
E-mail: celeste.cagnazzo@unito.it
ORCID: 0000-0003-4055-3139

Doi: 10.48286/aor.2021.02

KEY WORDS

Clinical trials; Covid-19; pandemic; disruption; recommendations.

IMPACT STATEMENT

The paper provides an overview of the actions taken to preserve clinical research during the pandemic, some of which it would be important to maintain in the future.

INTRODUCTION

Since the beginning of SARS-COV2 outbreak in Wuhan, more than 54 million people have been infected all around the world, reaching over a million deaths (1). The rapid spread of the COVID-19 pandemic posed an unprecedented challenge for healthcare and research systems, facing the worst crisis in the last 50 years (2).

In the early phase of the pandemic outbreak, Italy was the most affected European country: the first forced to a profound re-organization of the healthcare system, to not only effectively handle the pandemic but also keep the routine patient management and care. In this scenario, the research system had to provide scientifically useful and rapid answers, under an unprecedented media pressure.

HEALTHCARE HOSTAGE OF THE COVID-19

Providing care to patients has been extremely challenging, especially for oncologists.

Cancer patients infected with COVID-19 coronavirus have a 3.5 times increased risk of requiring admission in an intensive care unit (3); moreover, as most of adult Cancer Department resources are used to respond to the health emergency, patients are often treated in centres already suffering, due to the pandemic, from limited resources and instruments (4). In dedicated cancer centres, the general policy was to attempt to stay COVID-19 free, to ensure that enough clinical and intensive – care capacity could be reserved for critical cancer situations (5). An impossible goal for non-dedicated cancer institutions, to the point that international cancer societies decided to spread out priority driven guidelines for the management of onco-hematological patients during the emergency period (6).

READJUSTING CLINICAL TRIALS

The pandemic has caused a massive disruption in research worldwide; laboratories have closed, communications shut down, conferences cancelled and thousands of clinical trials (around 80% of non-COVID-19 ones) temporarily or permanently suspended. Furthermore, many researchers have been transferred, especially in the first months of the emergency, from clinical trials activity to operating in emergency and/or COVID-19 dedicated units (7).

A marked decline in screening and patient accrual has been detected (8), although in many cases only new recruitments were suspended, while already included patients continued to receive treatment thanks to alternative plans, jointly implemented by founders, institutions and regulatory authorities. This profound disruption has also affected the field of paediatric cancer, the early-phase clinical research above all (9).

During COVID breakdown, Pharmaceutical Industries and Sponsors made efforts to accelerate trial innovations providing digital tools thus allowing virtualization of certain processes to protect patient safety and trial integrity, also with support from regulatory guidelines (10). Simultaneously, Regulatory Agencies, first of all the Italian “Agen-

zia Italiana del Farmaco (AIFA)”, have implemented extraordinary measures to guide stakeholders in ensuring patient care and maintaining good data quality (11-13).

Indeed, we can pinpoint the main goals of these regulatory guidelines in clinical research being: the safeguard of patient safety, guaranteeing therapeutic continuity and the work ship of data integrity and consistency. Actual operating instructions are widely spread also with the help of scientific societies (14). New trials regarding COVID-19 infection were fast tracked, existing inefficiencies were promptly identified and streamlined, Good Clinical Practices (GCP) applied in a less conservative way, demonstrating that a reasonable balance between patient safety, regulatory burden, scientific quality and integrity may not be a utopia.

Patients remain central in any decision at all times from clinical to research activities, also during the adjustment of ongoing clinical trials. This centrality is the driving force behind various guidelines, leading especially in Italy, to the unhooking of rules that seem like engraved in stone.

Below, we highlight five innovation key points introduced by the guidelines for the management of clinical studies in the emergency period. Five notable reflections, perhaps worthy of being taken into consideration even in non-emergency times.

Telemedicine and activities outside the experimental site

Travel restrictions adopted during the pandemic led to delays and in some cases the impossibility for many patients to reach the trial sites at scheduled visits and laboratory or instrumental tests. In order to minimize the risk for patients to withdraw from treatment, regulatory authorities granted the possibility to perform blood tests, imaging or other diagnostic tests at the nearest local facility, provided it be certified as per national requirements.

This experience may be continued and integrated into clinical trial procedures, especially for the benefit of patients living far from specialized centres, at least for procedures not correlated to the primary endpoint of the study. Obviously if this practice becomes routine, valid mechanisms should be established to track and reimburse these extra institutional procedures. In addition, the staff of the peripheral centres should be adequately trained, and a clear division of responsibilities must be put in place under the supervision of the team, this be-

ing an objective easily achieved by strengthening the research networks (15).

Efforts to protect the patient have gone so far as to enable direct dispatch of oral medication from the hospital pharmacy to the patient's home, another practice that, after careful planning and adequate workforce, may become standard procedure.

The need for physical distancing to protect patients and research staff also motivated Countries to rapidly implement telemedicine programs (16). Telemedicine has already been around for 20 years, however before the pandemic it was mostly underused and in many cases hampered by administrative and/or bureaucratic barriers, such as the need for costly nationwide standardized payment policies, that ultimately prevented this approach from being incorporated into clinical practice or clinical studies (17). Having said that, many study-specific activities can be potentially provided through electronic tools: medical history collection, evaluation of quality of life, informed consent discussion and signature, re-consent and follow up visits are just some examples.

Decentralization and remote oversight

Another consequence of the restrictions imposed by the pandemic concerned the impossibility of carrying out on-site monitoring visits by CRA or Sponsor delegates, forcing replacement with alternative forms of oversight and monitoring.

Despite the fact that some forms of monitoring and especially of source data verification (e.g., use of Skype or Zoom) have currently been prohibited in many European Countries by local data protection policies, alternative ways such as mixed and risk-based systems, should not be excluded and could make way for large economic savings, all the while guaranteeing patient safety. This is especially true for studies promoted by non-profit organizations (18). There is no doubt that this evolution cannot occur without a general technological advancement of healthcare, especially in Italy, starting from electronic medical records, that are still far from being successfully or uniformly implemented.

Furthermore, remote work should become standard practice at least partially for audits and inspections, albeit maintaining certain activities that cannot be deferred on site.

A single ethical evaluation

The process adopted to quick start COVID-19 trials in Italy, which requires the approval by the Agen-

zia Italiana del Farmaco (AIFA) and that of only one Ethics Committee (EC), instead of every EC of each experimental centre involved, has significantly shrunk the timeline for authorization (14.1 ± 9.8 days rather than a mean of about 150 days). This was the biggest novelty for Italy, for a long time accustomed to a multitude of Ethics Committees and a redundancy of start-up procedures, often suffocating studies.

Considering the success of this approval process during the pandemic, it would be desirable to maintain it, also considering that this would finally bring us in line with the provisions of the European Regulation 536/2014 already fully implemented by other European Countries like Spain, and ultimately slashing costs of submission. Moreover, this could be applied to different types of clinical research, observational studies included.

Some concerns regarding this approach regard the possible overload for the Ethics Committee and the risk that a single ethical opinion might reduce the strictness of the evaluations. This being said, the current number of Ethics Committees existing in Italy (around ninety) and the number required by the Law 3/2018 should allow a not so onerous distribution of the authorization procedures.

Intermediate solutions could be evaluated, such as the establishment of a few highly specialized committees in the various areas of research, or in specific pathologies, which could be called upon depending on their expertise and on the subject of the experimentation, all the while guaranteeing that a national opinion is expressed.

Bureaucracy give way to science

The administrative burden of clinical research is a problem that has been alarming stakeholders (18) and contract and budget negotiations have been identified as time-consuming procedures interfering with study participation, to the point that the American Society of Clinical Oncology recommended the adoption of standardized contract templates (19).

Although in Italy the National Coordination Centre of Ethics Committees recently released a standard contract template for profit interventional studies, the negotiation process is still excessively centre-dependent and delayed by additional procedures imposed by the individual institutions and/or ethical committees.

In addition, there are still ongoing redundant procedures requiring periodic collection of identical

documents that could easily be shared between study promoters (e.g., curricula, trainings, certifications) and the reluctance to adopt simplifications that could save a lot of time and energy, trivially the use of the electronic signature.

On this subject, many exceptions have been granted during the pandemic thus enabling us to witness in Italy the kick-start of an academic study with the involvement of 600 centres in just 3 weeks. Note this was a prestigious study, evaluated by AIFA.

Finally, we ought to necessarily stop and consider how much work we have done so far was really useful in order for bureaucracy to give way to science once and for all.

No chance for research without professionals

The emergency period has emphasized how professionals dedicated to the management of the clinical trials and data collection, such as the Study Coordinator and Study Nurses, play a crucial role for the success of a clinical study, particularly in support of Investigators.

The current legislation requires their mandatory presence for phase 1 centres, and the Law 3/2018 imposes that “clinical trials of medicines make use of specific professionalism in the field of data management and research coordination”. However, these professional figures are substantially under-represented; adding to, a considerable heterogeneity in terms of education backgrounds, training and job descriptions, there is the of contractual stabilization and the lack of professional recognition at an institutional level (20, 21).

Nevertheless, there is ample evidence in literature that the presence of research infrastructures increases the performance of the centre and these are now mandatory, also given the whirling increase in the complexity of the research (22-24).

During the pandemic there was a deep gap between structures with strong infrastructures, which managed to keep their research projects going and even promote new ones, also thanks to

the possibility of implementing smart working (25), and small research centres which, having no adequate staff, were forced to stop clinical research activity all together.

Especially in view of at full application of European legislation, which will greatly increase the complexity in the management of studies for non-profit organizations, the stabilization of these resources can no longer be postponed (26).

PREPARING FOR THE FUTURE

The COVID-19 pandemic has caused an unparalleled global emergency, but at the same time it has triggered a profound analysis of ethics and research organization. Above all, it has allowed to make a way for a new vision of clinical research.

Clinical trials are an essential tool for scientific progress but COVID-19 has exposed aspects regarding their design and conduct that could be improved and simplified. Most trial aspects could be streamlined and modernized, and bureaucracy lightened without dramatic consequences at the expenses of research quality and consistency.

The innovations introduced during the pandemic by regulatory authorities have proved so successful that many stakeholders are clamouring to keep them even when the pandemic will be finally over (27-30).

Primarily Italy, which has always been pointed out as a slow Country with excessively cumbersome bureaucracy, through the joint work of Institution, regulatory authorities and stakeholders could recover its prestigious place in the world of research. The important lessons learned during the pandemic must not disappear at the end of the emergency.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

1. World Health Organization. Coronavirus disease (COVID-19) situation report. 2020. Available at: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports>. Accessed: 18/01/2021.
2. Emanuel EJ, Persad G, Upshur R, et al. Fair Allocation of Scarce Medical Resources in the Time of Covid-19. *N Engl J Med* 2020;382(21):2049-55.
3. Liang W, Guan W, Chen R, et al. Cancer patients in SARS-CoV-2 infection: a nationwide analysis in China. *Lancet Oncol* 2020;21(3):335-7.
4. Ueda M, Martins R, Hendrie PC, et al. Managing Cancer Care During the COVID-19 Pandemic: Agility and Collaboration Toward a Common Goal. *J Natl Compr Canc Netw* 2020;1-4.
5. van de Haar J, Hoes LR, Coles CE, et al. Caring for patients with cancer in the COVID-19 era. *Nat Med* 2020;26(5):665-71.
6. Tagliamento M, Lambertini M, Genova C, et al. Call for ensuring cancer care continuity during COVID-19 pandemic. *ESMO Open* 2020;5(3).
7. van Dorn A. COVID-19 and readjusting clinical trials. *Lancet* 2020;396(10250):523-4.
8. Ong MBH. Doroshow: NCI to accrue patients for COVID-19 longitudinal cohort. 2020.
9. Rubio-San-Simón A, André N, Cefalo MG, et al. Impact of COVID-19 in paediatric early-phase cancer clinical trials in Europe: A report from the Innovative Therapies for Children with Cancer (ITCC) consortium. *Eur J Cancer* 2020;141:82-91.
10. Xue JZ, Smietana K, Poda P, et al. Clinical trial recovery from COVID-19 disruption. *Nat Rev Drug Discov* 2020;19(10):662-663.
11. European Medicines Agency. Guidance On The Management Of Clinical Trials During The Covid-19 (Coronavirus) Pandemic. 2020. Available at: https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/guidanceclinicaltrials_covid19_en.pdf. Accessed: 18/01/2021.
12. Agenzia Italiana del Farmaco. Gestione degli studi clinici in Italia in corso di emergenza COVID-19 (coronavirus disease 19). 2020. Available at: <https://www.aifa.gov.it/-/gestione-degli-studi-clinici-in-italia-in-corso-di-emergenza-covid-19-aggiornamento-del-17-settembre-2020>. Accessed: 18/01/2021.
13. Food and Drug Administration . Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency. 2020. Available at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/fda-guidance-conduct-clinical-trials-medical-products-during-covid-19-public-health-emergency>. Accessed 25/11/2021.
14. Pinto C, Cagnazzo C. Indications regarding the management of interventional clinical trials with drugs during the current COVID-19 emergency in Italy. *ESMO Open* 2020;5(2).
15. Doherty GJ, Goksu M, de Paula BHR. Rethinking cancer clinical trials for COVID-19 and beyond. *Nat Cancer* 2020;1-5.
16. Hollander JE, Carr BG. Virtually Perfect? Telemedicine for Covid-19. *N Engl J Med* 2020;382(18):1679-81.
17. Men J. Lack of reimbursement barrier to telehealth adoption. 2015. Available at: <https://www.ajmc.com/view/lack-of-reimbursement-barrier-to-telehealth-adoption>. Accessed 18/01/2021.
18. Uren SC, Kirkman MB, Dalton BS, et al. Reducing clinical trial monitoring resource allocation and costs through remote access to electronic medical records. *J Oncol Pract* 2013;9(1): e13-16.
19. Thompson MA, Hurley PA, Faller B, et al. Challenges With Research Contract Negotiations in Community-Based Cancer Research. *J Oncol Pract* 2016;12(6): e626-32.
20. Cagnazzo C, Testoni S, Guarrera AS, et al. [Clinical research coordinators: a crucial resource.]. *Recenti Prog Med* 2019;110(2):65-7.
21. Martucci M, Guarrera A, Valente D, et al. Clinical research nurse in Italian centers: a mandatory figure?. *Recenti Prog Med* 2020;111(9):535-8.
22. Emanuel EJ, Schnipper LE, Kamin DY, et al. The costs of conducting clinical research. *J Clin Oncol* 2003;21(22):4145-50.
23. Davis AM, Hull SC, Grady C, et al. The invisible hand in clinical research: the study coordinator's critical role in human subjects protection. *J Law Med Ethics* 2002; 30(3):411-9.
24. Street A, Strong J, Karp S. Improving patient recruitment to multicentre clinical trials: the case for employing a data manager in a district general hospital-based oncology centre. *Clin Oncol (R Coll Radiol)* 2001;13(1):38-43.
25. Cagnazzo C, Franchina V, Maggiora P, et al. Revolutionizing clinical trial units in the pandemic era. *Ann Oncol* 2020; 31 (Suppl 4).

26. Cagnazzo C, Guarrera A, Cenna R, et al. Clinical research: enough players to get out there?. *Recenti Prog Med* 2019;110(6):285-91.
27. Cagnazzo C, Fagioli F. Bureaucracy gives way to science. What good the pandemic has left.. *Recenti Prog Med* 2020;111(10):565-7.
28. Lorusso D, Ray-Coquard I, Oaknin A, et al. Clinical research disruption in the post-COVID-19 era: will the pandemic lead to change? *ESMO Open* 2020;5(5).
29. Cagnazzo C, Besse MG, Manfellotto D, et al. Lessons learned from CoViD-19 for clinical research operations in Italy: what have we learned and what can we apply beyond? *Tumori* 2020. 9.
30. TransCelerate Biopharma INC. Beyond COVID-19: Modernizing Clinical Trial Conduct. 2020. Available at: https://www.transceleratebiopharmainc.com/wp-content/uploads/2020/08/TransCelerate_Beyond-COVID19_Modernizing-Clinical-Trial-Conduct_July-2020.pdf. Accessed: 18/01/2021.

OPINION PAPER

RESEARCHERS' PYRAMID. A NEW OPPORTUNITY FOR ITALIAN ONCOLOGY RESEARCH INFRASTRUCTURE?

S. Testoni ¹, R. Cenna ^{2,3}, A.S.E. Guarrera ⁴, M. Monti ¹, S. Stabile ⁵, I. Federici ⁶,
C. Taverniti ⁷, S. Pirondi ⁸, A. Ferrari ⁹, C. Cagnazzo ^{2,3}

¹ Biostatistics and Clinical Trials Unit, IRCCS Istituto Scientifico Romagnolo per lo Studio dei Tumori (IRST) Dino Amadori, Meldola, Italy

² Department of Public Health and Paediatrics, University of Turin, Turin, Italy

³ Paediatric Haematology-Oncology Department, Regina Margherita Children's Hospital, A.O.U. Città della Salute di Torino, Turin, Italy

⁴ Terapie Cellulari e Medicina Trasfusionale, AOU Careggi, Florence, Italy

⁵ Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milan, Italy

⁶ Clinica di Ematologia, AOU Ospedali Riuniti Umberto I, G.M. Lancisi, G. Salesi, Ancona, Italy

⁷ Oncologia Medica 1, Molinette Hospital, A.O.U. Città della Salute di Torino, Turin, Italy

⁸ Oncologia, AUSL Modena Area Sud Ospedale di Sassuolo, Sassuolo, Modena, Italy

⁹ Oncology Clinical Trial Office, SC Oncologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

CORRESPONDING AUTHOR:

Celeste Cagnazzo

Department of Public Health and Paediatrics

University of Turin

S.C. Oncoematologia Pediatrica

AOU Città della Salute e della Scienza

Presidio Ospedaliero Infantile Regina Margherita

piazza Polonia 94

10124 Turin, Italy

E-mail: celeste.cagnazzo@unito.it

ORCID: 0000-0003-4055-3139

Doi: 10.48286/aor.2021.03

ABSTRACT

The "Researchers' Pyramid" has represented the first main effort to formally recognize in Italy a specific category of professionals that, despite working in the healthcare field, has never been able to benefit from the stabilization options granted to the rest of the medical and healthcare staff. Stabilization of researchers by the Pyramid should in fact represent a chance to hire a large number of professionals that have been working in local pub-

lic IRCCS and IZS for a long time, like the Clinical Research Coordinators.

During the last trimester of 2019, we decided to start an investigation on the impressions of researchers about the new legislation, which introduced the researchers' Pyramid. This is a new path of 5 + 5 years thought to culminate with the stabilization of the researcher according to merit criteria, which should have ensured a big turning point compared to their working conditions. Three

months later, in the midst of a national emergency, we verified how much the reform staff had really impacted the working life of people who at that time should be totally dedicated to research against the virus.

Over half of respondents is optimistic regarding the actual benefit of the reform for employment stability. Over half (63.4%) of the “not optimistic respondents” considers the Pyramid a false path towards stabilization. Concerns were expressed in relation to the evaluation criteria during the ten-year period, considered by a third of the interviewed too exclusive and often not very suitable. Many individuals (41.5%) report the poor valoriza-

tion of personnel and much apprehension was recorded relating to the possibility of extending the reform to other institutes. Only 1412 of the over 35,000 potential beneficiaries have been hired.

The reform overall seems like an important opportunity for entry level or inexperienced personnel, a watered-down compromise for expert professionals. The fear conveyed from the great majority of the interviewed is that the pyramid is only a trick. It talks about a stabilization process, although it hasn't clarified how, after the ten-year period, this will take place. It also allows a partial solution of the problem in a very small share compared to the total number of clinical centers that do research.

KEY WORDS

Pyramid; researchers; stabilization; skills; precariousness.

IMPACT STATEMENT

The paper provides an overview of the researchers' impressions about the new legislation that should have ensured a big turning point compared to their working conditions.

BACKGROUND

The research in Italy is characterized by profound contradictions: great minds working in a system lacking economic resources and completely based on precariousness, as well as the total nonexistence of the researcher as a profession in the National Health System. A scenario that clashes with the application of the European Charter for Researchers principles (1): researchers as professionals; stimulating and well-equipped research environment and training centers; flexibility required to conduct research activities; stability of working conditions; adequate salary and social security and professional growth; geographic mobility, both intra-sectorial and between public and private centers; intellectual property right.

The only effort that in the recent years has been made in our country is a ministerial reform called “*Researchers' pyramid*”, a long reform work addressed to public cancer research and treatment (IRCCS) and zoo prophylactic institutes (IZS). Started in June 2016 with a mapping of all precarious staff (approximately 3800 people, 44 clinical research professional profiles, divided into two different categories: researcher and research sup-

porting professionals) (2) culminated in late 2019 with the approval, by the Council of Ministers, of the contract template signed by the Agency for the Negotiation Representation of Public Administrations (ARAN) and by the involved labor unions (3). The reform process collectively known as the “*Researchers' Pyramid*” (4-8) provides a 10 year working path: a 5-year, fixed-term subordinate employment contract, renewable for a further 5 years after a positive suitability assessment and in the presence of two types of conditions:

1. subjective: if the researcher is successfully evaluated based on productivity modalities and criteria;
 2. objective: if the Center has financial availability.
- The positive evaluation after ten years would enable, after verification of compliance with regulatory requirements, the inclusion of this new role in the National Health Service, in a way that still needs to be defined.

In order to favor “*veteran precarious*”, the personnel that by 31/12/2017 had accrued at least 3 years of seniority in the last 5 years were granted access to the new contracts without having to pass a new examination.

The commitment of the ministry has divided public opinion from the very beginning. On one hand, Institutions and the directors of institutes involved have always strongly defended it, describing it as a historical initiative that would have brought great advantages for the precarious personnel. On the other hand, researchers and the research personnel in time have become ever more hesitant in regards to its actual feasibility, denouncing major shortcomings in the contents of the reform (9-11). During the last trimester of 2019, unaware of how much the research scenario would have changed due to COVID-19, we decided to launch an investigation on the impressions of potential beneficiaries of the Researchers' Pyramid and to identify the critical elements of this reform. Three months later, in the midst of a national emergency, we verified how much the reform staff had really impacted on the professional life of people who at that time were completely dedicated to research on the virus. During this period of deep emergency, in fact, public opinion has restored a great deal of interest in clinical research and the key role of researchers. Particularly in Italy, deeply affected by the emergency, people have regained their trust towards the work of health professionals and have widely called upon the research field to make the ultimate effort. However, if on one hand this has prompted the competent authority to put in place a series of measures to try to speed up clinical trials and the use of effective drugs (12-14), on the other hand it has once again underlined the profound precariousness of Italian research.

METHODS

In September 2019, the GIDM (Gruppo Italiano Data Manager) shared with its members an online survey that could be completed anonymously, meant for the biomedical research personnel in Italy.

The questionnaire comprised of a descriptive section with a short summary of the main novelties introduced by the Researchers' Pyramid and a link to another page for more information, followed by different questions (binary or multiple choice, short answer, scoring question), divided in two sections:

- general – respondent's information: type of workplace, knowledge on the Ministry's initiative, general impressions on the topic;
- specific – respondent's evaluations on the single novelties introduced by the reform.

A copy of the survey is contained in available in **appendix 1-survey**.

Two semi-structured questionnaires were used as pilot to interview 10 researchers and 10 research assistants, coming from 5 institutes representing all the typologies foreseen by the questionnaire.

It is impossible to make a precise estimate of the study sample size, as GIDM members were given permission to spread the questionnaire among other colleagues. Considering the impossibility to define a priori a sample of respondents and considering the nature of the investigation, the decision was made to keep the survey open for 3 months and analyze the data, once more than 50 responses have been registered. Data were analyzed at the end of December.

In March 2020, a revision of the official documentation available was made to understand how many researchers and support staff had actually benefited from a stable contract, being able to "officially" work even during lockdown.

RESULTS

The questionnaire was completed by 147 respondents; the majority (n = 109, 74%) has declared to be already familiar with the Ministry's initiative. As for the direct seniors of the respondents, a large portion (n = 64, 43.5%) seems unaware of the reform and in many cases (n = 53, 36.1%) their knowledge on the subject is not reported.

The origin of the respondents is diverse, the majority being employed at public IRCCS/IZP (n = 78, 53.1%) or public Hospitals/University/Local Health Company (n = 50, 34.0%) (**figure 1**).

The respondent's profession was not included in those listed in the Pyramid in the minority of cases (n = 28, 19%), while for the greater part it corresponded to a profession included in the "Clinical Research Assistant" (n = 21, 55.1%) and "Clinical Researcher" categories (n = 38, 25.9%).

Regarding the actual benefit of the reform in terms of employment stability, over half of the respondents declared to be optimistic (Group A: n = 101, 68.7%). Of the remaining share (Group B), almost all (Group C, n = 41, 89.1%) were willing to provide three main reasons why the Pyramid would not be an adequate solution (**figure 2**).

Over half of the sample of the Group C (n = 26, 63.4%) considers the Pyramid a false path towards employment stabilization, leading to a prolunga-

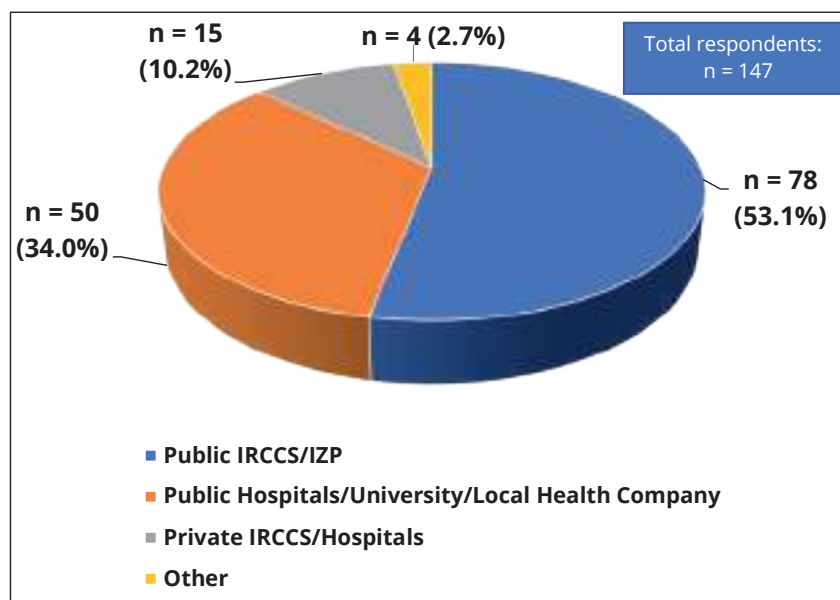


Figure 1. Workplace of respondents.

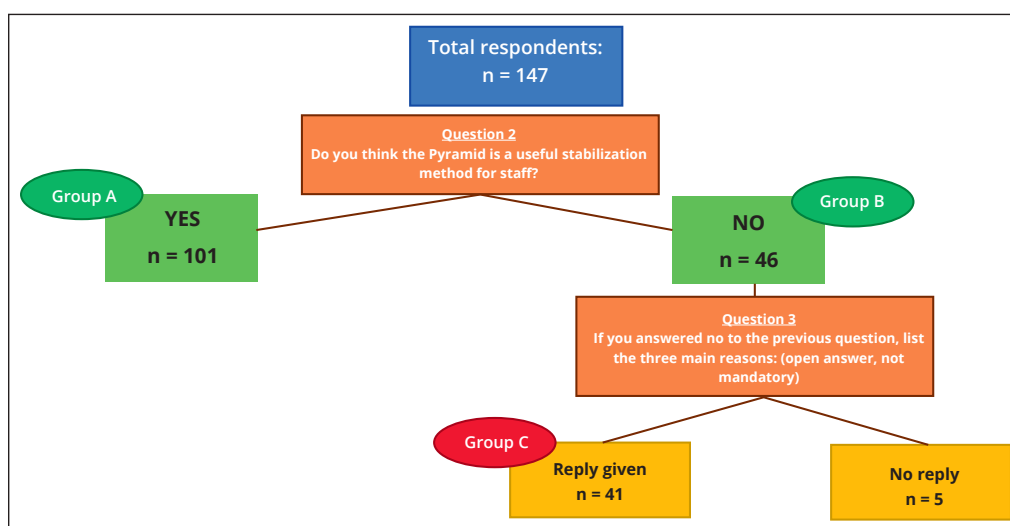


Figure 2. Impressions regarding the real usefulness of the reform for staff stabilization.

tion of the precarious status, and, as highlighted by 15% of the interviewed ($n = 7$), without even providing real motivations on the process of inclusion at the end of the ten-year period in the Pyramid. Concerns were also expressed in relation to the evaluation criteria during the ten-year period, considered by a third of the interviewed ($n = 11$) to be too exclusive and often not very suitable for the assigned role. Many individuals report ($n = 17$, 41.5%) the poor personnel recognition, due to inadequate pay and lack of a managerial-process. Much apprehension was recorded relating to the possibility of extending the reform to the non

IRCCS/IZS public institutes ($n = 4$, 9.8%), the actual feasibility and sustainability of the system ($n = 2$, 4.9%), and the accuracy of the established criteria to access the pyramidal course ($n = 2$, 4.9%). Returning to the total sample, when called to give a vote between 1 (low) and 10 (high) on the extent of the pyramid as the solution, even if partially, to precariousness, the average vote was 5.5, with most respondents having an intermediate opinion (scored 5: $n = 28$, 19%; scored 6: $n = 26$, 17.7%). Collected votes regarding the actual possibility to extend the reform to public institutes showed a similar average result (5.2), while more optimism

POTENTIAL EXTENSION	SCORE N (%)									
	1	2	3	4	5	6	7	8	9	10
PUBLIC INSTITUTES	18 (12.4)	12 (8.3)	11 (7.6)	11 (7.6)	24 (16.6)	19 (13.1)	19 (13.1)	10 (6.9)	5 (3.4)	16 (11.0)
IRCCS/PRIVATE HOSPITALS	15 (10.2)	6 (4.1)	3 (2.0)	12 (8.2)	21 (14.3)	22 (15.0)	28 (19.0)	17 (11.6)	7 (4.8)	16 (10.9)

Table I. Possibility of extending the reform to other institutions.

emerged in relation to the potential implementation of the pyramid system in private IRCCS and hospitals (average score 6.6) (**table I**).

Of those working in public IRCCS/IZS, more than half ($n = 45$, 53.8%) was granted access to the pyramidal stabilization process, 29 (37.2%) were cut out, and a smaller portion denied having knowledge in the matter ($n = 7.9\%$). The most common reason for being excluded was the lack of necessary prerequisites ($n = 14$, 48.3%) (**figure 3**).

In terms of the specific employment categories described in the reform, the respondents have not expressed a clear-cut position: a little over half of them ($n = 88$, 59.9%) considers it consistent with their educational and professional background, while the remaining portion holds an opposite position. Among the given reasons for the latter, the following stand out: a) the flattening of the

background compared to that of the majority of potential beneficiaries (39% of respondents), b) the inadequate remuneration (33.9%), and c) the impossibility to pursue a managerial position. The question as to why the possibility to pursue a managerial position was not included is shared among all respondents: 92.5% ($n = 146$) does not agree.

When having to say with a score from 1 (low) to 10 (high), to what extent the contract suggested by the Pyramid elevates the professional figures included, the respondents expressed an intermediate opinion with an average score of 5.5.

As for the remuneration, focusing on those respondents working for public IRCCS/IZS that entered the stabilization process, the majority ($n = 25$, 59.5%) considers their future wage will decrease compared to their current one, a large portion ($n = 15$, 35.7%) believe it will not be subject to

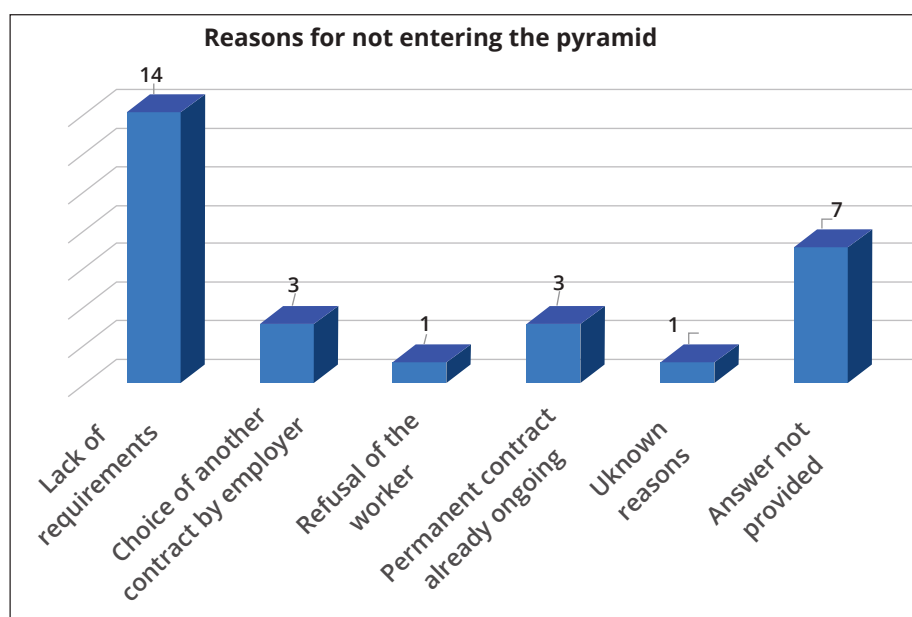


Figure 3. Reasons for not entering the pyramid.

substantial differences, while only two were confident on an increase in wage.

A wide range of responses was collected regarding to the degree of possibility that, following the ten-year process outlined by the Pyramid, the personnel would be in fact stabilized, with only a minor portion of respondents ($n = 23$, 15.6%) convinced that chances for this to occur could be over 50% (**figure 4**).

With the first (and for now only) hiring phase, 1412 professionals were hired (five-year contract), for a total of 31 institutions involved (**figure 5**).

DISCUSSION

This reform program led by the Ministry, the “Researchers’ Pyramid”, has represented the first main effort to formally recognize a specific category of professionals that, despite working in the healthcare field, has never been able to benefit from the stabilization options granted to the rest of the medical and healthcare staff. Stabilization of researchers has in fact always been a privilege for few, de facto unattainable for those research pro-

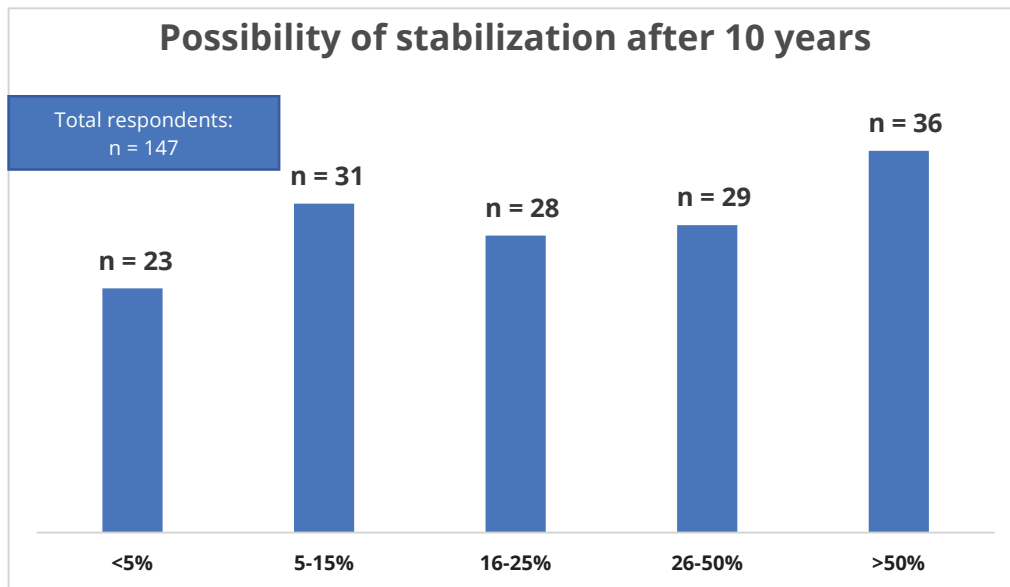


Figure 4. Possibility of stabilization after 10 years.



Figure 5. Number of professionals hired (five-year contract).

files considered “atypical”, like that of the Clinical Research Coordinators.

This process pictured by the Pyramid should represent, provided it is indeed achievable and financially sustainable, a chance to hire a large number of professionals that have been working in local public IRCCS and IZS for a long time.

However, despite the great emphasis given to this initiative by politics and some of institutes' directors, most of the potential beneficiaries and trade unions have bitterly criticized it in terms of content, highlighting many critical points. A first major inconsistency are the criteria to access the Pyramid: the fact that in order to benefit from the reform it is required for the professional to have accrued 3 years of seniority in the last 5 years in the same workplace, has de facto cut out many professionals that, despite being able to count up to decades of seniority will not be recognized with the latter because accrued under a different contract type that excludes a dependent relationship (VAT, scholarships) and/or neutralized by several contract interruptions. This data is confirmed in our research, according to which the lack of the prerequisites is the most common reason for failed access to Pyramidal system. Another critical aspect lies in the indicators that should be used for the periodical renewal according to the pyramidal system. The first drafts of the decree necessary to clear out this aspect had from the start reflected very restrictive prerequisites, conflicting with the possibilities pyramidal professionals were offered, de facto seeming much more restrictive compared to those that were currently used to evaluate the existent executive directors (managerial profiles), who ultimately will remain greatly privileged both on a professional categorization level and on a financial one, unlike the beneficiaries of the reform. This reform would increase the despised 3 approach of “Publish or Perish”, so to speak, that already underlies clinical research. Moreover, the publication indicator is not applicable with most profiles included in the Pyramid (clinical research coordinators, budget and contracts office and library staff) who's main focus is far from that of publishing scientific papers. The final version of the decree, published while the survey was still available for completion online, has ultimately confirmed these concerns, by indicating very restricting prerequisites, particularly for the clinical researcher's profile.

The investigation has also confirmed the inadequacy of the professional status in respect to the educational and professional background of the poten-

tial beneficiaries. The two categories established by the reform, despite including seniority upgrades, will never equal those of current health managers. This aspect is in contrast with the curricula of the personnel identified by the Ministry that, often, in addition to the decennial experience acquired on the job, has been recognized with prestigious academic titles, such as specialization courses and/or PhDs. An understatement of abilities is a dangerous risk reported by the respondents and already highlighted in the past by several professionals. A decrease in salary is yet another risk for many of those who have access to the pyramidal system that will have to face a reduction of their salary compared to their current one, to the point that it's preferable not to access the pyramidal system.

The professional classification also calls for attention that, in both categories envisioned by the Ministry, is very specific: in case of absence of an executive position in the pyramid, professionals would end up to with supervisors that could possibly be lacking necessary skills to supervise.

The reform, overall, seems like an important opportunity for entry level or inexperienced personnel, a watered-down compromise for expert professionals.

The numbers regarding the implementation of the reform are also not very encouraging: 1412 people are undoubtedly a small group compared to the totality of professionals who have been waiting for a real contract, often for decades.

Indeed, not less important, the fear conveyed from the great majority of the interviewed and already notified by groups of this field: Pyramid talks about a stabilization process, although it has not clarified how, after the ten-year period, this will take place. For this reason, for now, as most professionals put it, it is all a matter of procrastination of the issue, with most of them believing only a minimal portion will be indeed stabilized. In fact, industry associations often remember 1412 people hired for now have only a five-year contract in their hands, very different from the chimera of the indeterminate contract advertised by Institutions (15). It will be very interesting to investigate with future work on the percentage of actual reconfirmations in the 10 years foreseen by the pyramid and above the share, and modalities with which this staff will officially and permanently become an integral part of the National Health System.

A delicate issue remains unresolved: the reproducibility.

Assuming that these issues will be resolved and

that the permanent stabilization of public IRCCS and IZS will become reality, there is still great skepticism in relation to the possibility of extending this initiative to private IRCCS/Institutes. Particularly regarding to the extension to public hospitals, universities and ASL that, despite not having been contemplated by the Ministry, keep on representing an important research source, both basic and clinical, and that to this day are still subject to unsustainable employment loss. The number of centers involved in clinical research in Italy is close to two thousand, a much larger number than the share of institutions that can benefit from the reform. Does the staff in these centers have less of a right to consider research a job?

Meanwhile, the virus continues to circulate and there is more and more talk on research. Perhaps the time has become to consider it a real job, not just a passion.

By the end of 2019, the rapid spread of the new severe acute respiratory syndrome (SARS) coronavirus (CoV), named SARS-CoV-2 or 2019-nCoV (16-18), made Italy one of the most affected countries: with 37 860 confirmed cases and 4032 deaths according to the data of Istituto Superiore di Sanità on 20th of March 2020 (19). The arrival of the pandemic has put a strain on our nation, from many points of view. Firstly on our National Health System, already strongly weakened by years of continuous cuts, poor investments and little attention

from politics to the point that Nature denounced it on February 2018 (20).

On an economic level, with a very long lockdown period and, no less important, on an organizational and psychological level, with the life of health workers completely out of whack (21-25).

Even the biologist who first isolated the virus in Italy is a precarious worker; a reality that has greatly stirred public opinion coming to terms with a problem well known among experts, that had been pointed out for years. "Underpaid excellences", 3,500 precarious workers make Italian research great", newspapers wrote (26).

How can these "ghost professionals" contribute to the battle towards COVID, particularly now that as non-employees their access to the hospital / research centers is denied?

In full awareness of not being able to formally suggest a revision of the law, we would like to underline the most critical aspects being: i) the lack of a concrete career possibility for researchers, ii) the absence of salary adequate to the level of education and the skills acquired, iii) the total uncertainty about what can happen to the researcher at the end of the 10-year period foreseen by the pyramid.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

1. Commission Recommendation of 11st of March 2005 on the European Charter for Researchers and on a Code of Conduct for the Recruitment of Researchers.
2. Ministero della Salute - Direzione Generale della Ricerca e dell' Innovazione in Sanità. La figura del bibliotecario e la piramide dei ricercatori. 2018. Available at: http://www.bibliosan.it/ftp/ws2018/Luciani_WS2018.pdf.
3. Ministero della Salute. Comunicato n. 72 11 giugno 2019 Ricerca, via libera del Consiglio dei ministri al contratto per il personale degli Irccs. Available at: http://www.salute.gov.it/portale/news/p3_2_4_1_1.jsp?lingua=italiano&menu=salastampa&p=comunicatistampa&id=5204.
4. Presidente della Repubblica. L Decreto Legislativo 15 giugno 2015, n. 81 Disciplina organica dei contratti di lavoro e revisione della normativa in tema di mansioni, a norma dell'articolo 1, comma della legge 10 dicembre 2014, n. 183. Disciplina organica dei contratti di lavoro e revisione della normativa in tema di mansioni, a norma dell'articolo 1, comma 7, della legge 10 dicembre 14, n. 183. Gazzetta Ufficiale della Repubblica Italiana 2015.
5. Presidente della Repubblica. Legge 11 dicembre 2016, n. 232: Bilancio di previsione dello Stato per l'anno finanziario 2017 e bilancio pluriennale per il triennio 2017-2019. Gazzetta Ufficiale della Repubblica Italiana 2016.
6. Presidente della Repubblica. Legge 27 dicembre 2017, n. 205: Bilancio di previsione dello Stato per l'anno finanziario 2018 e bilancio pluriennale per il triennio 2018-2020. Gazzetta Ufficiale della Repubblica Italiana 2017.
7. Presidente della Repubblica. Legge 30 dicembre 2018, n. 145 Bilancio di previsione dello Stato per l'anno finanziario 2019 e bilancio pluriennale per il triennio 2019-2021. Gazzetta Ufficiale della Repubblica Italiana 2019.
8. Presidente della Repubblica. Decreto-Legge 30 dicembre 2019, n. 162: Disposizioni urgenti in materia di proroga di termini legislativi, di organizzazione delle pubbliche amministrazioni, nonché di innovazione tecnologica. Gazzetta Ufficiale della Repubblica Italiana 2019.
9. Direttori generali IRCCS pubblici. Ecco perché la "Piramide dei Ricercatori" valorizza chi fa ricerca. La posizione dei Direttori scientifici degli Irccs pubblici. Quotidiano Sanità. 2019. Available at: https://www.quotidianosanita.it/lettere-al-direttore/articolo.php?articolo_id=73296.
10. Associazione Ricercatori in Sanità. Piramide del ricercatore, è il momento di disperare? 2019. Available at: https://www.quotidianosanita.it/lettere-al-direttore/articolo.php?articolo_id=73227.
11. Associazione Ricercatori in Sanità. Se la "piramide dei ricercatori" finisce per umiliare chi fa ricerca 2019. Available at: https://www.quotidianosanita.it/lettere-al-21direttore/articolo.php?articolo_id=7322.
12. Agenzia Italiana del Farmaco. Gestione degli studi clinici in Italia in corso di emergenza COVID-19 (coronavirus disease 19) (Versione 2 del 7 aprile 2020).
13. Agenzia Italiana del Farmaco. Gestione degli studi clinici in Italia in corso di emergenza COVID-19 (coronavirus disease 19) (12/03/2020). 2020.
14. Presidente della Repubblica. Decreto Legge n. 23 dell'8 aprile 2020. Gazzetta Ufficiale della Repubblica Italiana 2020.
15. Associazione Ricercatori in Sanità. Piramide del ricercatore. Terminata la prima fase di reclutamento 2020. Available at: https://www.quotidianosanita.it/lettere-al-direttore/articolo.php?articolo_id=73227.
16. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020;382(8):727-33.
17. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020;395(10224):565-74.
18. World Health Organization. Available at: <https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19-11-march-2020>.
19. Istituto Superiore di Sanità. Available at: <https://www.epicentro.iss.it/coronavirus/sars-cov-2-sorveglianza-dati>.
20. Abbott A. Italian election leaves science out in the cold. *Nature* 2018;554(7693):411-2.
21. Bersano A, Pantoni L. On being a neurologist in Italy at the time of the COVID-19 outbreak. *Neurology* 2020.
22. Remuzzi A, Remuzzi G. COVID-19 and Italy: what next? *Lancet* 2020;395(10231):1225-8.

23. Grasselli G, Pesenti A, Cecconi M. Critical Care Utilization for the COVID-19 Outbreak in Lombardy, Italy: Early Experience and Forecast During an Emergency Response. JAMA 2020.
24. Lambertini M, Toss A, Passaro A, et al. Cancer care during the spread of coronavirus disease 2019 (COVID-19) in Italy: young oncologists' perspective. ESMO Open 2020;5(2).
25. Pietrantonio F, Garassino MC. Caring for Patients With Cancer During the COVID-19 Outbreak in Italy. JAMA Oncol 2020.
26. La Repubblica. Eccellenze sottopagate, sono 3500 i precari che fanno grande la ricerca italiana 2020. Available at: https://www.repubblica.it/cronaca/2020/02/04/news/_eccellenze_sottopagate_sono_3_500_i_precari_che_fanno_grande_la_ricerca_italiana-247585350/.

APPENDIX 1-SURVEY

Section one

1. Were you already aware of this Ministry initiative?
 - a.** Yes
 - b.** No
2. Do you think it is a useful stabilization method for staff?
 - a.** Yes
 - b.** No
3. If you answered no to the previous question, list the three main reasons: (open answer).
4. To what extent do you think it may be a solution, albeit partial, for the problem of precariousness in the research sector?
(score from 1-low, to 10-high).
5. To what extent do you think this initiative can be transferred to private IRCCS or hospitals?
(score from 1-low, to 10-high).
6. To what extent do you think this initiative can be transferred to non-IRCCS institutions?
(score from 1-low, to 10-high).
7. Does your profession fall into one of the two profiles indicated in the contract?
 - a.** Yes, Researcher
 - b.** Yes, Professional Health Research Collaborator
 - c.** No
8. In what type of facility do you work?
 - a.** Public IRCCS/IZS
 - b.** Private IRCCS/Hospital
 - c.** Public Hospital/University/ Local health Company
9. If you work in a Public IRCCS / IZP, have you entered the stabilization path envisaged by the Pyramid?
 - a.** Yes
 - b.** No
10. If you have not entered the stabilization path, can you indicate the reason? (open answer).

Section two

1. Based on your role and job description, do you consider the professional category foreseen in the contract (D special / D) to be appropriate?
 - a.** Yes
 - b.** No
2. If you answered no to the previous question, please indicate the 3 main reasons (open answer).
3. How much do you think that the contract proposed by the pyramid adequately enhances the professional figures it frames?
(score from 1-low, to 10-high).
4. In what percentage do you think that, at the end of the ten-year path envisaged by the contract, the staff will be effectively stabilized?
 - a.** < 5%
 - b.** 5-15%
 - c.** 16-25%
 - d.** 26-50%
 - e.** > 50%
5. The Pyramid has 3 contribution brackets, but excludes a management path. Do you think it correct?
 - a.** Yes
 - b.** No
6. If you work in a public IRCCS / IZP and are part of the stabilization process, you believe that the new contract will be, on a salary basis:
 - a.** Disadvantageous compared to the previous one
 - b.** Similar to the previous one
 - c.** Advantageous compared to the previous one
7. Are its managers aware of the innovations introduced by the Pyramid?
 - a.** Yes
 - b.** No
 - c.** I don't know

RESEARCH ARTICLE

EGFR⁺ GLIOBLASTOMA STEM CELLS TARGETING BY CD16^{158V}-CHIMERIC RECEPTOR T CELLS AND CETUXIMAB

C. Cenciarelli ¹, S. Caratelli ¹, R. Arriga ², G. Lanzilli ¹, A. Ottaviani ¹, T. Sconocchia ³, G. C. Spagnoli ^{1,4}, A. Venditti ⁵, G. Iezzi ⁴, M. Roselli ², D. Lauro ², H. E. Marei ⁶, G. Sconocchia ¹

¹ Institute of Translational Pharmacology, CNR, Rome, Italy

² Department of Systems Medicine, Endocrinology and Medical Oncology, University of Rome Tor Vergata, Rome, Italy

³ Otto Loewi Research Center, Chair of Immunology and Pathophysiology Medical University of Graz, Graz, Austria

⁴ Department of Surgery, Ente Ospedaliero Cantonale, Università della Svizzera Italiana, Lugano, Switzerland

⁵ Department of Biomedicine and Prevention, Hematology, University of Rome Tor Vergata, Rome, Italy

⁶ Department of Cytology and Histology, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

CORRESPONDING AUTHOR:

Carlo Cenciarelli
Institute of Translational Pharmacology
Department of Biomedical Sciences
National Research Council (CNR)
via Fosso del Cavaliere 100
00133 Rome, Italy
E-mail: carlo.cenciarelli@ift.cnr.it
ORCID: 0000-0001-7480-4608

Doi: 10.48286/aor.2021.04

ABSTRACT

Glioblastoma multiforme (GBM) is the deadliest human brain tumor with a median survival following diagnosis of 14–16 months. Innovative therapeutic approaches are urgently needed. Cancer stem cells (CSC) from GBM resist current chemo- and radiotherapies and can generate recurrent and aggressive tumors. To envisage innovative therapeutic approaches of potential clinical use, we engineered T cells with Fcγ-chimeric receptors (CRs) to elicit antibody-dependent cellular cytotoxicity (ADCC) in the presence of mAbs specific for tumor associated antigens (TAA). Indeed, in previous studies, we successfully redirected CD16^{158V}-CR T cells against KRAS-mutated colorectal carcinoma cells. Since surface overexpression of epidermal growth

factor receptor (EGFR) is frequently detectable in GBM, we assessed, *in vitro*, the anti-GBM potential of polymorphic CD16-CR T cells, in combination with anti-EGFR mAbs, on GBM-derived EGFR⁺ CSC. Our results indicate that CD16^{158V}, but not CD16^{158F}-CR engineered T cells incubated with cetuximab, but not panitumumab, induced the elimination of GBM-derived CSC through a caspase-3 dependent mechanism, and produced high amounts of TNFα and IFNγ upon recognition of target cells. These data pave the way towards pre-clinical development of innovative GBM treatments, taking advantage of CD16^{158V}-CR engineered T cells and therapeutic antibodies.

ABBREVIATIONS

ADCC: antibody-dependent cellular cytotoxicity; **CAR**: chimeric antigen receptor; **CR**: chimeric receptor; **CSC**: cancer stem cells; **EGFR**: epidermal growth factor receptor; **FcγR**: IgG constant fragment receptor; **FGF2**: fibroblast growth receptor 2; **FITC**: Fluorescein isothiocyanate; **GBM**: glioblastoma multiforme; **IL-7**: interleukin-7; **IL-15**: interleukin-15; **INF**: interferon-gamma; mAb: monoclonal antibody; NK: natural killer; **PBMC**: peripheral blood mononuclear cells; **PDGFR**: platelet-derived growth factor receptor; **PE**: Phycoerythrin; **TNFα**: tumor necrosis factor-alpha.

KEY WORDS

CD16-chimeric receptors; ADCC; cetuximab; panitumumab; EGFR; Glioblastoma; cancer stem cells.

IMPACT STATEMENT

We present an innovative therapeutic strategy in which anti-EGFR monoclonal antibody, cetuximab redirects CD16^{158V}-chimeric receptor T cells against GBM stem cells, these findings may support a potential role of CD16-CR T cell-based immunotherapy in the management of EGFR+ GBM.

INTRODUCTION

Glioblastoma multiforme (GBM), high-grade glioma, has an annual incidence of 3-4 and 7 cases per 100,000 people in Europe and the USA, respectively (1). Median onset age is of 63 years and median survival after clinical diagnosis is of 14-16 months. In many cancer types, the bulk of tumor cells derive from small populations of cancer stem cells (CSC), also known as tumor-initiating cells (2). CSC are characterized by their ability to successfully seed new tumors when implanted in low numbers into experimental animals. Such cells may persist in recurrent GBM due to their enhanced resistance to chemotherapy and radiotherapy (2-6). In previous studies, we identified two types of CSC in several human GBM clinical specimens, hereafter referred to as core GBM (c-CSC) and peritumor tissue-derived CSC (p-CSC) (3-5). C-CSC are characterized by a higher proliferative potential *in vitro*, correlating with a higher tumor-initiating ability *in vivo*, as compared to p-CSC (5).

WHO 2016 classification, highlighting a large number of genetic alterations associated with specific GBM phenotypes, has improved the classical histological classification (6, 7). The Cancer Genome Atlas (TCGA), subdivides these tumors into three subclasses based on the pattern of expression and genetic alterations: classical/EGFR+, proneural/PDGFR+ and mesenchymal/NF1+ classes (4). EGFR wild type (wt) is overexpressed in almost 50% of GBM, whilst its activating mutation (EGFRvIII) has an overall prevalence of almost 60% in patients whose tumors show amplification of EGFR wt. Enhanced

EGFR activity leads to activation of downstream signaling pathways such as Raf/MEK/Erk and PI3K/Akt pathways, which are responsible for the malignant phenotype of glioma (8). Another subset of gliomas, the PDGFR subclass account for 25-30% of GBM, and is characterized by dysregulation of PDGFR activity, which in some cases is due to amplification and rearrangements of the PDGFRα gene locus, and in others to overexpression of the PDGF ligands (9). In previous studies, we showed that EGFR and PDGFR targeting decreases GBM invasiveness (3, 4) whereas shRNA inhibition of either PDGFRα or PDGFRβ signaling induces apoptosis of GBM stem cells (3, 10).

In the last decade monoclonal antibodies (mAbs), targeting tumor markers and immunological checkpoints inhibitors, have successfully entered clinical practice. As a consequence, they are now included in standard treatment protocols (6, 7). Moreover, adoptive transfer of chimeric antigen receptor (CAR) transduced T cells, recognizing markers expressed on tumor surfaces, is being increasingly utilized (11). Currently, CAR-T cell therapy is approved for B-cell lymphoma and leukemia treatment and its potential relevance for GBM is actively being investigated (12-14).

Fcγ-chimeric receptors (Fcγ-CRs) may share the same transmembrane (TM) and intracellular chimeric signaling domains of "conventional" CAR-T cells. However, while the latter express extracellular domains including a single-chain variable

fragment (ScFv) specific for a marker located on the surface of tumor cells, Fcγ-CRs express the extracellular portion of the FcγRs (15, 16). Fcγ-CR T cell-based immunotherapy has been designed to transfer antibody-dependent cellular cytotoxicity (ADCC) function of innate immune cells including NK cells to T lymphocytes (17-19). The rationale of using Fcγ-CR T cells rather than NK cells is based on evidence indicating that: 1) T cells can be easily expanded *in vitro*, and effectively infiltrate the tumor microenvironment (TME); 2) tumor infiltration by T lymphocytes is usually associated with a favorable prognosis (20); and 3) upon conjugation with cancer cells, NK cells undergo apoptosis (21) and CD16 and NK cell activating receptor down-regulation (11, 22). In contrast, the role of NK cells in solid tumor is unclear since they barely infiltrate the TME and may not be directly associated with favorable prognosis (23).

FcγRs are classified into three major groups: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16), widely distributed on the surface of innate immune cells. CD64 is the only high-affinity receptor binding monomeric IgG molecules, whereas CD32 and CD16 are low-affinity receptors that bind weakly to monomeric IgG. CD16 polymorphisms do influence their binding affinity to IgG (18, 19).

Based on this background, here we assessed the ability of T cells expressing polymorphic CD16-CRs in combination with EGFR-specific therapeutic mAbs to prevent the expansion of GBM-derived EGFR⁺ c-CSC.

MATERIALS AND METHODS

Antibodies and reagents

Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD3, allophycocyanin (APC)-conjugated mouse anti-human CD3, phycoerythrin (PE)-conjugated mouse anti-human CD16, FITC-conjugated mouse anti-human CD107a, PE-conjugated mouse anti-human EGFR, mouse anti-human CD3, and mouse anti-human CD28 were purchased from BD Bioscience (San Jose, CA). Rabbit anti-EGFR and rabbit anti-caspase-3 were purchased from Cell Signaling Technology (Leiden, The Netherlands). Rabbit anti-PARP-1 was from Santa Cruz Biotechnology (Santa Cruz, CA), and mouse anti-β-actin from SIGMA-Aldrich (Saint Louis, MO). Rabbit anti-mouse and donkey-anti-rabbit peroxidase-conjugated secondary antibodies were

purchased from Jackson Immunoresearch Laboratories (West Grove, PA). Cetuximab (Erbix 5 mg/mL, Merck, Darmstadt, Germany) and panitumumab (Vectibix 20 mg/mL, Amgen, Thousand Oaks, CA) were commercially available. Chemiluminescence HRP substrate was purchased from Millipore (Burlington, MA), while Retronectin (Recombinant Human Fibronectin) was obtained from Takara Bio (Saint-Germain-en-Laye, France). Monensin sodium salt was purchased from SIGMA-Aldrich (Saint Louis, MO). Human recombinant interleukin-7 (IL-7), interleukin-15 (IL-15), epidermal growth factor (EGF), and fibroblast growth factor 2 (FGF2), were purchased from PeproTech (London, UK). Interferon-gamma (INFγ) and tumor necrosis factor-α (TNFα) were obtained from Thermo Fisher Scientific (Waltham, MA) while 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from SIGMA-Aldrich (Saint Louis, MO). Bio-Rad Protein Assay was obtained from Bio-Rad (München, Germany), NuPAGEBis-Tris gels from Invitrogen, (Carlsbad CA), Hybond-P Extra membrane from Amersham Biosciences (GE Healthcare Life Science-Buckinghamshire, UK), and Z-VAD-FMX (Biomol) from Enzo Life Science (Farmingdale NY). Nonidet-P40 (NP-40), sodium dodecyl sulfate (SDS), Tris-base, NaCl, EDTA, sodium orthovanadate (Na₃VO₄), and protease inhibitors cocktail, were all purchased from SIGMA-Aldrich (Saint Louis MO). Iscove Modified Dulbecco Media (IMDM), Fetal Bovine Serum (FBS), RPMI 1640 medium, L-glutamine, and penicillin/streptomycin were obtained from Thermo Fisher Scientific (Waltham, MA). A 1:1 mixture of 10X Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) was obtained from Invitrogen (Carlsbad, CA) and matrigel from SIGMA-Aldrich (Saint Louis, MO). Mycoplasma detection kit was purchased from Minerva Biolabs (Berlin, Germany), and Accutase and GeneJuice Transfection Reagent from Millipore (Burlington, MA).

Cell cultures

Primary T cells were expanded in RPMI 1640 supplemented with 10% FBS, IL-7 (10 ng/mL), and IL-15 (5 ng/mL). The generation and growth of GBM-derived c-CSC cultures were described previously (3-5). Briefly, c-CSC were grown as floating tumor spheres in serum-free DMEM/F12 (1:1), supplemented with 20 ng/mL EGF, 10 ng/mL FGF2 and containing L glutamine 2 mM, glucose 0.6%, putrescine 9.6 μg/mL, progesterone 0.025 mg/mL, sodium selenite 5.2 ng/mL, insulin 0.025 mg/mL,

apo-transferrin sodium salt 0.1 mg/mL, sodium bicarbonate 3 mM, Hepes 5 mM, BSA 4 mg/mL, heparin 4 µg/mL (all purchased by Sigma-Aldrich). For flow cytometry, cytotoxicity, MTT assays and Western blots, medium-sized tumor spheres were dissociated in single-cell suspensions by Accutase (Millipore, Burlington, MA) at 37 °C and plated onto matrigel pre-coated dishes. The GBM-derived c-CSC cultures (# 2, 3, 4) are primary cells with a limited life span. First, following 30 passages, the proliferation rate of the cells is increasingly reduced ending up to cell cycle arrest. Second, small tumor spheres display a necrotic phenotype. These cells are kept in culture only for the time necessary to perform the experiments. The HEK293T packaging cell line (RRID: CVCL_0063) grown in IMDM supplemented with 10% FBS, was used to produce helper-free retrovirus for T cell transduction. All experiments were performed with mycoplasma-free cells.

Retrovirus production and T cells transduction

The method of transfection of HEK293T cells was reported previously (18, 19). Briefly, HEK293T packaging cells were transfected using GeneJuice Transfection Reagent with a Peg-Pam vector containing the Moloney murine leukemia virus (MoMLV) gag-pol genes, the RDF vector carrying RD114 envelope, and the SFG retroviral vector harboring CD16^{158F}-CR or CD16^{158V}-CR.

Peripheral blood mononuclear cells (PBMC) at a 0.5×10^6 cells/mL concentration were activated in 24-wells plates pre-coated with mouse anti-human CD3 and anti-CD28 mAbs. The viral supernatant was loaded on retronectin-coated 24-wells plates and activated PBMC were then seeded on the retrovirus loaded-plate for 72 hours at 37 °C under 5% CO₂.

MTT viability assay

The MTT viability assay was performed as described previously (18, 19). Briefly, the viability of GBM-derived c-CSC incubated with CD16^{158F}-CR or CD16^{158V}-CR T cells and cetuximab (C) or panitumumab (P) was assessed at 48 hours. Non-transduced T cells (NT) were used as experimental control. c-CSC (10^4 cells/well) were plated on matrigel pre-coated 96-well flat-bottom plates the day before. CD16^{158F}-CR T cells were added to target cells at a 4:1 effector/target (E/T) and CD16^{158V}-CR T cells at a 2:1 E/T cell ratio, with or without mAbs (3 or 6 µg/ml). Five independent experiments were performed in quadruplicates.

Flow cytometry

CD16-CR transduction efficiency on T cells was assessed by flow cytometry with PE-conjugated mouse anti-human CD16 and FITC-conjugated mouse anti-human CD3. Analysis of EGFR expression on c-CSC was carried out with PE-conjugated mouse anti-human EGFR. Cells were analyzed by a 2-laser BD FACSCalibur (Becton Dickinson, S. Jose, CA) flow cytometer, and results were analyzed by utilizing the Tree Star, Inc. (San Carlos CA) FlowJo software.

Cytotoxicity assay

To assess the cytotoxic potential of transduced lymphocytes, c-CSC (#3, 4) seeded as single cells (2×10^5) onto matrigel pre-coated 24-well dishes, were left 1 day in proliferation medium before to be incubated for 4 hours at 37 °C under 5% CO₂ in the presence or absence of CD16^{158V}-CR T cell at a 2:1 E/T cell ratio, with or without cetuximab (3 µg/mL), while NT were used as control. Z-VAD-FMX pan-caspase inhibitor was used at 50 µM and added to target cells 1 hour before starting the cytotoxicity assay. After removal of supernatants and washing with PBS, c-CSC were detached with Trypsin-EDTA solution. Subsequently, cells were resuspended with Annexin V binding buffer (500 µL), for each tube, 500 µL solution was splitted in 250 µL for blank tube and 250 µL for the tube with FITC-conjugated-Annexin V, PI, APC-conjugated anti-human CD3. Finally, cells were incubated 30 minutes at room temperature and analyzed using flow cytometry by gates posted on FSC-H high cells and CD3 negative.

Cytotoxic degranulation assay

To assess the secretion of lytic granules following CD16^{158V}-CR T cells cross-linking with target cells, CD107a expression was investigated on the membrane of CD16-CR T cells by flow cytometry. c-CSC (#3, 4) were plated as single cells (5×10^4) onto matrigel pre-coated 96-well dishes, and CD16^{158V}-CR T cells were added at 2:1 E/T cell ratio with or without cetuximab and 5 µL FITC-conjugated anti-CD107a. After 1 h incubation at 37 °C under 5% CO₂, 2 µM Monensin was added for 4 h. After incubation, plates were centrifuged a 1.200 rpm for 5 min, supernatants gently discarded and 100 µL of PE-conjugated-anti-CD16 added. After 30 min incubation, cells were washed and fixed with 150 µL of 1% paraformaldehyde (PFA)-PBS directly added on the dishes. As positive control of secretion of lytic granules, CD16^{158V}-CR T cells were cross-linked with plastic bound human anti-CD3. Finally, CD16^{158V}-CR

T cells were collected and transferred in tubes for assessing CD16 and CD107a expression by cytometric analyses.

ELISA

Cell culture supernatants of c-CSC (#2, 3, 4), incubated with CD16^{158F}-CR T cells or CD16^{158V}-CR T cells with or without cetuximab (C) or panitumumab (P) were collected at 48 hours, as described in MTT assay. INF γ and TNF α secretion levels were measured by ELISA according to the manufacturer's instructions.

Western blots analysis

GBM-derived EGFR⁺ c-CSC seeded as single cells (5×10^5) onto matrigel pre-coated well dishes were left 1 day in proliferation medium prior to a 4 hours incubation with CD16^{158V}-CR T cells or NT at a 2:1 E/T cell ratio, with or without cetuximab. Afterwards, T cells were removed and c-CSC were collected in 200 μ l of lysis buffer (1% NP-40, 0.01% SDS, 20 mM Tris-HCl pH 7.4, 300 mM NaCl, 1 mM EDTA, 1 mM Na₃VO₄ and a protease inhibitors cocktail, including AEBSEF, Aprotinin, Bestatin, Leupeptin, and Pepstatin A. Cells were then sonicated with two pulses of 5 sec at 50% of amplitude (Sonics and Materials, Newtown, CT). Equal amounts (30 μ g/lane) of total protein extracts, determined by Bio-Rad Protein Assay, were loaded on NuPAGE Bis-Tris gels and transferred on Hybond-P Extra membrane. Filters were immunoblotted using the following primary antibodies: rabbit anti-EGFR, rabbit anti-caspase-3, rabbit anti-PARP-1, and mouse anti- β -actin. After three washings with TBS-Tween buffer, immuno-reactive proteins were detected using rabbit anti-mouse or donkey-anti-rabbit peroxidase-conjugated secondary antibodies directed to the appropriate primary antibodies. Proteins were then visualized using the chemiluminescence HRP substrate. Gels and Images acquisition was done by HP Photosmart Essential Ver. 1.12 and Adobe Photoshop CS5 respectively. The densitometric analyses of protein bands normalized against to β -actin protein levels were performed using the ImageJ software (NIH, USA).

Statistical analysis

Statistical analysis was performed with Prism5 (Graph Pad) and Microsoft Office Excel 2019. Data shown are representative of results obtained from three or five independent experiments carried out in quadruplicates, as detailed in the specific sec-

tions. The results were analyzed by two-way ANOVA and Bonferroni's post-tests. Data are expressed as mean $\pm$ standard deviation (SD) and P values $\leq$ 0.05 were considered statistically significant.

RESULTS

We first assessed T cell transduction efficiency of CD16^{158F}- and CD16^{158V}-CRs over time by flow cytometry. As reported in **figure 1 a**, 9.49%, 18.5%, and 23.0% of T cells were efficiently transduced with CD16^{158F}-CR at day 6, 9 and 16, respectively, as compared to 20.7%, 30.5%, and 43.2% for CD16^{158V}-CR transduced T cells. Cumulative data referred to transduced T lymphocytes derived from five different healthy donors are reported in **figure 1 b**. Considering the different transduction efficiency of the two viral vectors, to reliably evaluate, in a comparative manner, the effector potential of either transduced T cell populations, cytotoxicity assays were carried out using two effector/target (E/T) cell ratios: 4:1 and 2:1 for CD16^{158F}- and CD16^{158V}-CR T cells, respectively.

The cell surface expression of the EGFR on the three c-CSC (# 2, 3, and 4), under investigation, was analyzed by flow cytometry (**figure 1 c**). Although all cells expressed EGFR, a lower MFI (26.0) was observed for c-CSC 2, as compared to c-CSC 3 (56.6) and c-CSC 4 (44.5). These results are consistent with previously reported gene expression and Western blot data (3).

The anti-tumor potential of transduced T cells against c-CSC targets, in the presence or absence of therapeutic mAbs, was comparatively assessed following 48 hours co-culture by using the MTT viability assay (**figure 2 a-c**). CD16^{158V}-CR T cells significantly decreased the viability of all target c-CSC in the presence of IgG1 mAb cetuximab. In contrast, IgG2 mAb panitumumab was unable to mediate cytotoxic activity and CD16^{158F}-CR T cells were similarly ineffective in the presence or absence of either anti EGFR reagent. In parallel experiments, we observed that amounts of INF γ (**figure 2 d-f**) and TNF α (**figure 2 g-i**) released in culture supernatants, were significantly higher in cultures performed in the presence of CD16^{158V}-CR T cells and cetuximab, as compared with those from cultures including CD16^{158F}-CR and cetuximab, or NT cells. IgG2 mAb panitumumab was ineffective in triggering cytokines release by transduced T cells (**figure 2 d-i**).

To corroborate these data, the induction of apoptosis in target cells, following four hours incu-

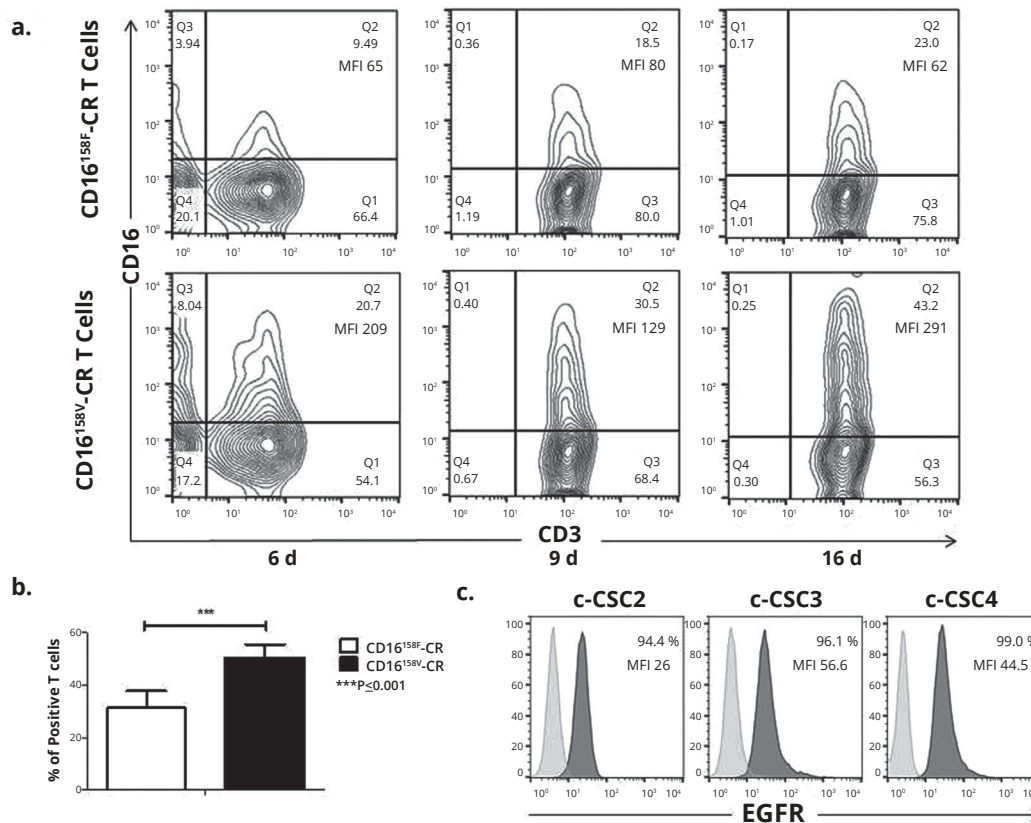


Figure 1. Efficiency of CD16-CR expression on transduced T lymphocytes and EGFR surface expression on GBM-derived c-CSC.

a. A representative experiment of CD16^{158F}-CR (upper panel) and CD16^{158V}-CR (lower panel) surface expression in transduced T cells, as observed in a time-course experiment at 6, 9, 16 days after PBMC activation. Percentages of CD3⁺/CD16⁺ T lymphocytes are reported in the upper right quadrants of the contour plots. The mean fluorescence intensity (MFI), indicating the average density of CD16 surface expression, is reported for each time point. Cells were double stained with PE-conjugated mouse anti-human CD16 and FITC-conjugated mouse anti-human CD3. CD16 staining was carried out on transduced T cells at least three times with similar results. **b.** The histogram shows the cumulative percentage of positive T lymphocytes for CD16^{158F}- and CD16^{158V}-CR 16 days after viral infection of cells from five healthy donors; ***P value ≤ 0.001 is highly significant. **c.** EGFR surface expression on GBM-derived c-CSC. Data refer to a representative experiment assessing % of EGFR expression on c-CSC 2 (94.4%), c-CSC 3 (96.1%) and c-CSC 4 (99.0%) plated on pre-coated matrigel dishes. Cells are visualized with PE-conjugated mouse anti-human EGFR versus isotype-stained cells. EGFR staining was carried out at least three times with similar results. The MFI is displayed below the (%) EGFR positivity for each GBM-derived c-CSC.

bation in the presence of effector cells and mAbs, was also evaluated by flow-cytometry. Cultures including c-CSC 3 or c-CSC 4 target cells were harvested and stained with APC-anti-CD3, FITC-annexin V, and Propidium Iodide (PI). Among c-CSC 3 (**figure 3 a**) and c-CSC 4 (**figure 3 b**) we observed 33.1% and 16.7% of Annexin V⁺ cells, respectively, following incubation with CD16^{158V}-CR and cetuximab. ADCC was caspase-dependent since it was inhibited by the pan-caspase inhibitor Z-VAD-FMX (**figure 3 a, b**). Accordingly, Western blot experiments documented caspase-3 and PARP1 cleavage in both c-CSC 3 and c-CSC 4 target cells (**figures 3 c, d**), following incubation with CD16^{158V}-CR T cells and cetuximab, whereas EGFR expression was not modified (**figure 3 c, d**).

In order to estimate the levels of cleaved caspase-3 and PARP1 proteins, a quantitative densitometric analysis of the proteins bands by using ImageJ software was performed. We reported more than 10 and 5 fold increase of caspase-3 and PARP1 cleavage relative to β -actin levels in c-CSC3, respectively (**figure 3 d**). Similar results were observed in c-CSC 4 with 5 fold increase of caspase-3 and PARP1. No signal was quantified in the lanes with the application of pan-caspase inhibitor Z-VAD-FMX (**figure 3 d**). Variations observed in EGFR protein levels were not statistically significant.

To demonstrate that CD16^{158V}-CR T cells induced target cells cytotoxicity by exocytosis of perforin and granzyme-containing granules, we assessed the expression of CD107a, a sensitive marker of CD8

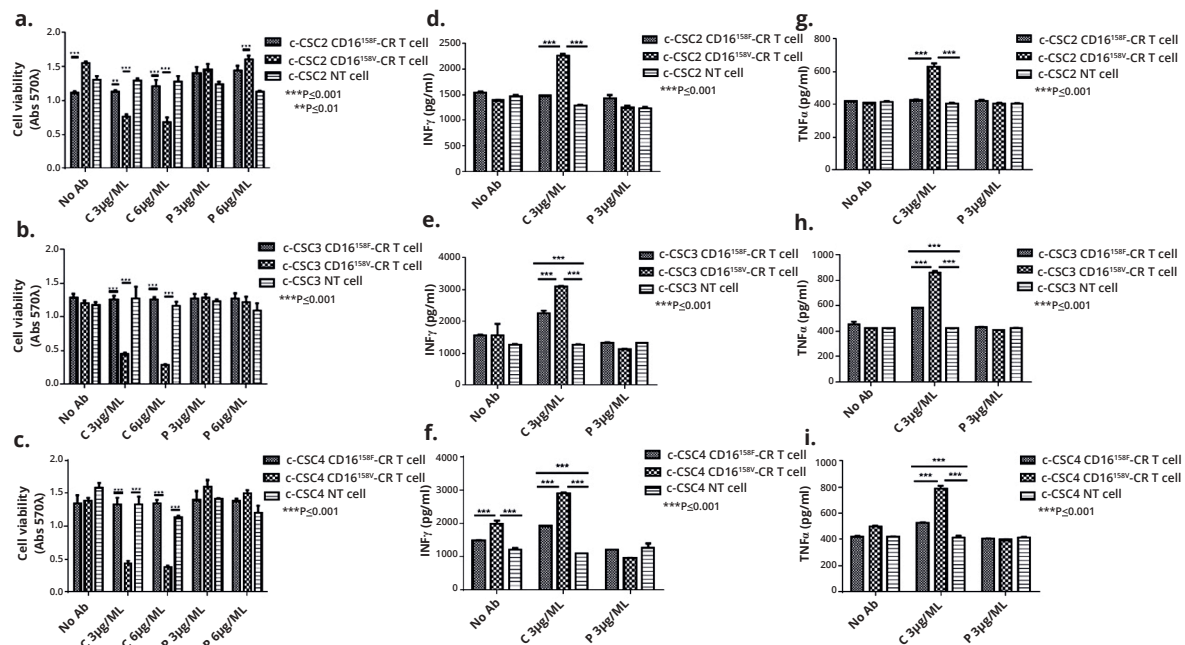


Figure 2. Co-culture of CD16-CR engineered T cells and GBM-derived c-CSC in combination with cetuximab reduced c-CSC viability and induced significantly INF γ and TNF α secretion.

a, b, c. Histograms show viability, as measured by MTT assay, of c-CSC (#2, 3, 4) cells, following 48 hours incubation with CD16^{158F/V}-CR T cells in the presence or absence of anti-EGFR mAbs at different concentrations (3, 6 μ g/mL). No effect on c-CSC survival was recorded using non-transduced T cells (NT) in the presence or absence of cetuximab (C) or panitumumab (P). 4:1 and 2:1 E/T cell ratios were used for assays with CD16^{158F}-CR and CD16^{158V}-CR T cells, respectively. The figure reports cumulative data, with mean $\pm$ (SD) and ***P values $\leq$ 0.001, **P values $\leq$ 0.01 related to c-CSC (#2, 3, 4) viability, as observed by using effector cells from five different donors in independent experiments. **d, e, f.** CD16^{158V}-CR engineered T cells in combination with cetuximab induce significant INF γ and TNF α secretion. Histograms refer to INF γ release, as detected in the culture supernatants of c-CSC (#2, 3, 4) after 48 hours incubation with CD16^{158F}-CR- and CD16^{158V}-CR T cells or NT in the presence or absence of cetuximab (C) or panitumumab (P) (3, 6 μ g/mL). **g, h, i** Histograms refer to TNF α release in culture supernatants of c-CSC (#2, 3, 4) after 48 hours incubation with CD16^{158F}-CR- and CD16^{158V}-CR T cells or NT in the presence or absence of cetuximab (C) or panitumumab (P). 4:1 and 2:1 E/T cell ratios were used for assays with CD16^{158F}-CR and CD16^{158V}-CR T cells, respectively. The figure reports cumulative data, with mean $\pm$ (SD) and ***P values $\leq$ 0.001, from ELISA assays from experiments performed by using effector cells from five different donors in independent experiments.

positive T cell degranulation following activation. Flow cytometry analysis did not detect any CD107a surface expression by CD16^{158V}-CR T cells upon conjugation with target cells and cetuximab (figure 4, middle and lower panel). In contrast, activation of CD16^{158V}-CR T with plastic bound anti-CD3 antibody, promoted mobilization of CD107a on T cells surface membrane (figure 4, right upper panel).

DISCUSSION

Recent studies support the safety and effectiveness of intraventricular and intra-tumoral CAR-T administration for the treatment of CNS malignancies (24-26). Donovan *et al.*, evaluated the efficacy of the loco-regional administration into the cerebrospinal fluid of EPHA2, HER2 and IL13R α 2 CAR-T cells (24).

This therapeutic approach was validated in pre-clinical models for the treatment of ependymoma and primary, metastatic and recurrent medulloblastoma. These results provided the basis for extending the same treatment modality in humans. Brown *et al.*, evaluated the safety and efficacy of CAR-T directed against IL13R α 2 for the treatment of recurrent GBM through a loco-regional intraventricular administration (27). A primary goal for the success of CAR-T based-immunotherapy in brain tumors and in solid tumors should envisage strategies for regulating T lymphocytes trafficking in the parenchyma. Baron *et al.* in the early 1993, have showed as T cells expressing alpha 4 integrin are able to leave blood and enter the brain parenchyma, overcoming the blood-brain barrier (28). More recently, Zhu *et al.* reported that T cells infiltration into the brain parenchyma may occur in the presence of an

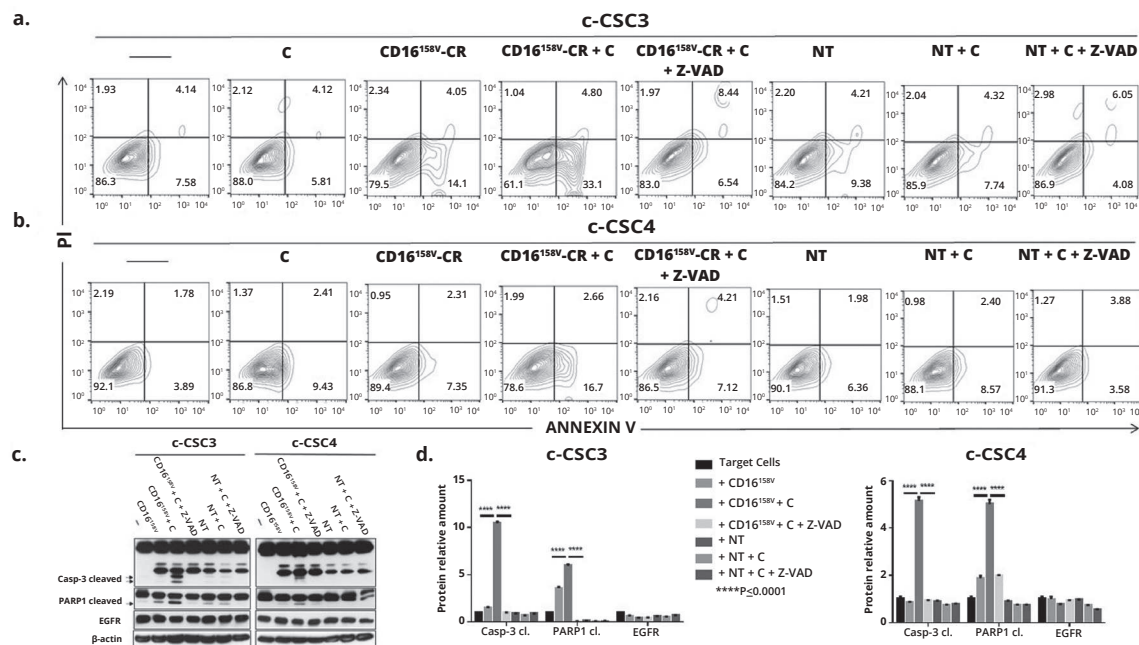


Figure 3. CD16^{158V}-CR engineered T cells in combination with cetuximab induce ADCC of c-CSC by caspase-3 activation.

a, b. These panels report flow cytometry data of a representative experiment of cytotoxicity using c-CSC 3 (**a**) and c-CSC 4 (**b**) target cells in the presence of CD16^{158V}-CR T cells with 3 μ g/mL of cetuximab (C). After 4-5 hours incubation, cells were harvested, stained with APC-anti-CD3, FITC-annexin V and propidium iodide (PI), and analyzed by flow cytometry. Data are representative of three experiments independently performed and show a significant increase of apoptotic cells in the presence of cetuximab, as indicated (%) in the lower right quadrants. Cell death is blocked by 50 μ M of pan-caspase inhibitor Z-VAD. No effect was recorded using non-transduced cells (NT) in the presence or absence of cetuximab. A 2:1 E/T cell ratio was used. **c.** CD16^{158V}-CR engineered T cells in combination with cetuximab (C) induce apoptosis of c-CSC 3 and c-CSC 4. c-CSC were incubated with CD16^{158V}-CR T cells plus cetuximab (C); after 4 hours incubation, cells were harvested and protein extracts immunoblotted with anti-caspase-3, anti-PARP1, and anti-EGFR antibodies. Western blots analyses show caspase-3 activation and PARP-1 cleavage induced by CD16^{158V}-CR T cells in the presence of cetuximab. The pan-caspase inhibitor Z-VAD blocks caspase-3 and PARP1 cleavages, but EGFR expression is not affected. No effect was recorded using NT in the presence or absence of cetuximab. A 2:1 E/T cell ratio was used. **d.** Histograms report a quantitative protein analysis of Caspase-3, PARP-1 and EGFR relative to β -actin protein levels in c-CSC 3 and c-CSC 4. Data shown are representative of three experiments independently performed. ****P value ≤ 0.0001 is highly significant.

altered TME such as the induction of the chemokine CXCL10 in the glioma site in IFN- α and IFN γ dependent manner. The blockade of CXCL10 with a specific mAb abrogated the efficient glioma homing of cytotoxic T lymphocytes (CTL) (29). Importantly, overexpression and/or enhanced activity of EGFR and its variant (EGFRvIII) have frequently been reported in human GBM (4, 26). Since high EGFR/EGFRvIII expression designates an aggressive GBM subtype, these markers do represent attractive targets for immunotherapeutic approaches (30). In previous work, we have generated CD16^{158F/V}-CR DNA constructs expressing phenylalanine (F) or valine (V) at position 158, efficiently promoting effector functions upon transduction into T lymphocytes on breast cancer and colorectal carcinoma cells (18,19). Here, we have addressed the possibility to target

GBM-derived EGFR⁺ c-CSC by using CD16^{158F/V}-CR engineered T cells and specific therapeutic mAbs. Cetuximab is a human-murine chimeric IgG1, instead panitumumab is a fully human IgG2. Several reports have described, both *in vitro* and *in vivo*, how these mAbs are able to elicit ADCC (17-19). The effects of the ligation of the Fc portion of the mAbs with the Fc receptors on the cells depend on the specificity of the Fc receptors for a given IgG class (31). For instance, CD16-CR T cells bind cetuximab (IgG1) upon recognition of the ligand on target cells, but do not bind soluble mAbs (18, 19). Thus, GBM-derived c-CSC are targeted by CD16^{158V}-CR T cells and cetuximab treatment, with timing and effectiveness comparable to those observed for long term-established tumor cell lines (18). In contrast, panitumumab mAb (IgG2) preferably binds to CD32-CR rather than CD16-CR T cells, and

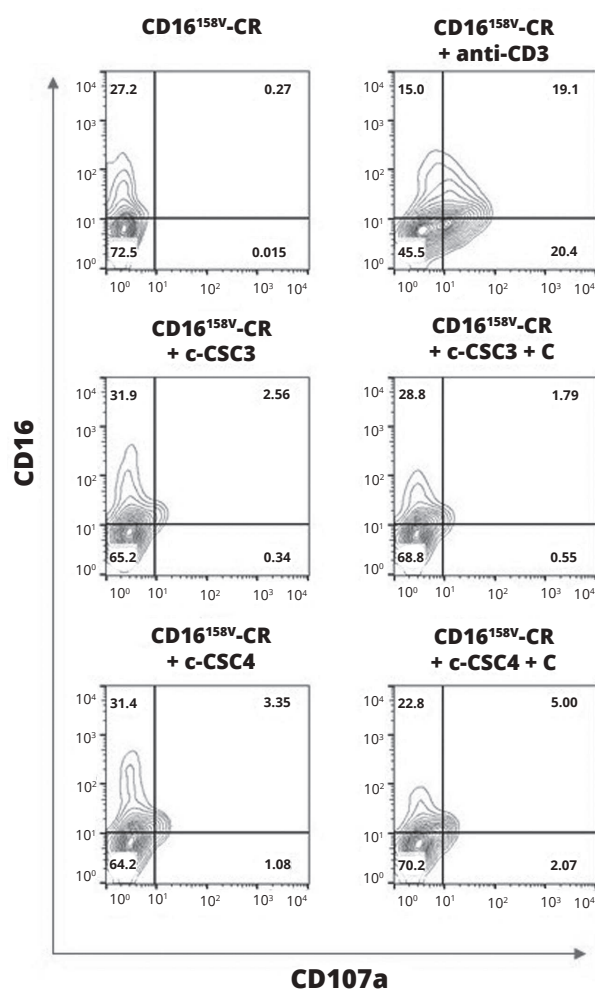


Figure 4. Cross-linking of CD16^{158V}-CR engineered T cells with c-CSC and cetuximab does not induce T cells degranulation.

The panel reports the mobilization of CD107a on engineered T cells surface assessed by flow cytometry. c-CSC3 and c-CSC4 target cells were incubated with CD16^{158V}-CR T cells in presence or without 3 µg/mL of cetuximab (C) and anti-CD107a-FITC. After 5 hours incubation, CD16^{158V}-CR T cells were further incubated with anti-CD16-PE. The upper panel (right upper quadrant) reports the positive control as displayed by CD107a expression (19.1%) on CD16^{158V}-CR engineered T cells activated with plastic bound anti-CD3 antibody. The middle and lower panel show the lacking of positivity for CD107a in the presence of cetuximab (C) after incubation of effector cells with c-CSC3 or c-CSC4 (right upper quadrants). A 2:1 E/T cell ratio was used. Data shown are representative of five experiments independently performed.

therefore no target cells cytotoxicity was recorded in our context in presence of panitumumab. mAb subclasses critically impacted on the effectiveness of CD16-CR T cells mediated-ADCC (16, 18). Our results are in line with what it has been shown by other investigators where Fcγ-CRs polymorphisms influenced the binding of engineered T cells to tumor cells coated with IgG (16, 18, 19).

Here, we demonstrate the lacking of CD107a expression accounting for defective degranulation, (32, 33) but CD16^{158V}-CR T cells generate fully competent immune response including INFγ and TNFα secretion and target cells cytotoxicity as reported here and elsewhere (17). This led us to assume the engagement of cell death surface receptors such as Fas/Fas ligand and TRAIL-R/TRAIL in mediating target cells elimination (17). Therefore, in our future investigations we aim to clarify more thoroughly the mechanisms underlying the GBM cancer stem cells elimination triggered by CD16^{158V}-CR T cells with cetuximab.

CONCLUSIONS

GBM is characterized by dismal prognosis, and there is an urgent need for innovative therapeutic approaches. Current GBM treatment is based on the use of temozolomide, a DNA alkylating agent. However, resistant tumor cell subsets promoting recurrence, rapidly emerge. Although these cells, presenting CSC features, do frequently express EGFR, treatment with small molecules targeting the EGFR signal transduction pathway has proven largely disappointing, thus suggesting that direct immune-mediated targeting of this marker could be more effective (34). Our data indicate that, indeed, expansion of GBM-derived c-CSC may be prevented by ADCC mediated by CD16^{158V}-CR T cells and anti-EGFR IgG1 mAb.

While the *in vitro* nature of our study represents an obvious limitation, these findings nevertheless underline the high potential relevance of this therapeutic approach and pave the way towards *in vivo* pre-clinical investigations.

ACKNOWLEDGMENTS

We thank Dr. Matilde Paggiolu and Dr. Pamela Papa for their administrative assistance.

ETHICAL STATEMENT

Procedures for collection of adult human GBM-derived CSC were approved by the Ethical Committee of the Catholic University of Rome, as reported previously (4, 5). Informed consent was obtained, and all patients were fully aware of the aims of this

work. The ethical principles of the declaration of Helsinki were strictly followed.

H.E.M. collected the data and revised the statistical analysis. All authors read and approved the final edited manuscript.

AUTHORS CONTRIBUTIONS

G. S. conceived this study, supervised the experiments and edited the manuscript; C.C. performed the MTT viability assays, drafted and edited the manuscript; S.C. and A.O. contributed to the execution of the transfection and infection experiments, and Western blots; G.L. performed the flow cytometry for CD16/CD107a and EGFR. T.S. assisted to do the ELISA; R.A. and D.L. performed the cytotoxicity assays by flow cytometry; G.I., G.C.S., A.V., M.R. contributed to the final editing of the manuscript;

FUNDINGS

Associazione Italiana per Ricerca sul Cancro (AIRC). Grant number: IG17120; Ministero Istruzione, Ricerca e Università - Consiglio Nazionale delle Ricerche (MIUR-CNR). Grant number: DSB.AD013.002.021.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

- Heynckes S, Daka K, Franco P, et al. Crosslink between Temozolomide and PD-L1 immune-checkpoint inhibition in glioblastoma multiforme. *BMC Cancer* 2019;19(1):117.
- Gimple RC, Bhargava S, Dixit D, et al. Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer. *Genes Dev* 2019;33(11-12):591-609.
- Cenciarelli C, Marei HE, Felsani A, et al. Correction: PDGFR α depletion attenuates glioblastoma stem cells features by modulation of STAT3, RB1 and multiple oncogenic signals. *Oncotarget* 2017;8(22):36931.
- Cenciarelli C, Marei HE, Zonfrillo M, et al. PDGF receptor alpha inhibition induces apoptosis in glioblastoma cancer stem cells refractory to anti-Notch and anti-EGFR treatment. *Mol Cancer* 2014;13:247.
- Lama G, Mangiola A, Proietti G, et al. Progenitor/Stem Cell Markers in Brain Adjacent to Glioblastoma: GD3 Ganglioside and NG2 Proteoglycan. *J Neuropathol Exp Neurol* 2016;75(2):134-47.
- Kamran N, Alghamri MS, Nunez FJ, et al. Current state and future prospects of immunotherapy for glioma. *Immunother* 2018;10(4):317-39.
- Adhikaree J, Moreno-Vicente J, Kaur AP, et al. Resistance Mechanisms and Barriers to Successful Immunotherapy for Treating Glioblastoma. *Cells* 2020;9(2). pii:E263.
- Gan HK, Kaye AH, Luwor RB. The EGFRvIII variant in glioblastoma multiforme. *J Clin Neurosci* 2009;16:748-54.
- Dai C, Celestino JC, Okada Y, et al. PDGF autocrine stimulation dedifferentiates cultured astrocytes and induces oligodendrogliomas and oligoastrocytomas from neural progenitors and astrocytes in vivo. *Genes Dev* 2001;15:1913-25.
- Kim Y, Kim E, Wu Q, et al. Platelet-derived growth factor receptors differentially inform intertumoral and intratumoral heterogeneity. *Genes Dev* 2012;26:1247-56.
- Marei HE, Althani A, Caceci T, et al. Recent perspective on CAR and Fcy-CR T cell immunotherapy for cancers: Preclinical evidence versus clinical outcomes. *Biochem Pharmacol* 2019;166:335-46.
- CE, Starr R, Aguilar B, et al. Stem-like Tumor-Initiating Cells Isolated from IL13Ra2Expressing Gliomas Are Targeted and Killed by IL13-Zetakine-Redirected T Cells. *Clin Cancer Res* 2012;18(8):2199-209.
- Morgan RA, Johnson LA, Davis JL, et al. Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. *Human gene therapy* 2012;23:1043-53.
- Ahmed N, Brawley V, Hegde M, et al. HER-2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma: a phase I dose escalation trial. *Jama Oncol* 2017;3(8):1094-101.
- Clémenceau B, Congy-Jolivet N, Gallot G, et al. Antibody-dependent cellular cytotoxicity (ADCC) is mediated by genetically modified antigen-specific human T lymphocytes. *Blood* 2006;107:4669-77.
- Kudo K, Imai C, Lorenzini P, et al. T lymphocytes expressing a CD16 signaling receptor exert antibody-dependent cancer cell killing. *Cancer Res* 2014;74(1):93-103.
- D'Aloia MM, Caratelli S, Palumbo C, et al. T lymphocytes engineered to express a CD16-chimeric antigen receptor redirect T-cell immune responses against immunoglobulin G-opsonized target cells. *Cytother* 2016;18(2):278-90.
- Arriga R, Caratelli S, Lanzilli G, et al. CD16-158-valine chimeric receptor T cells overcome the resistance of KRAS-mutated colorectal carcinoma cells to cetuximab. *Int J Cancer* 2019;146(9):2531-8.
- Caratelli S, Arriga R, Sconocchia T, et al. In vitro elimination of epidermal growth factor receptor-overexpressing cancer cells by CD32A-chimeric receptor T cells in combination with cetuximab or panitumumab. *Int J Cancer* 2020;146(1):236-47.
- Däster S, Eppenberger-Castori S, Hirt C, et al. Absence of myeloperoxidase and CD8 positive cells in colorectal cancer infiltrates identifies patients with severe prognosis. *Oncoimmunol* 2015;4(12):e1050574.
- Sconocchia G, Arriga R, Tornillo L, et al. Melanoma cells inhibit NK cell functions. *Cancer Res* 2012;72(20):5428-9.

22. Caratelli S, Sconocchia T, Arriga R, et al. FCy Chimeric Receptor-Engineered T Cells: Methodology, Advantages, Limitations, and Clinical Relevance. *Frontiers in immunol* 2017; 8:457.
23. Sconocchia G, Zlobec I, Lugli A, et al. Tumor infiltration by FcyRIII (CD16) + myeloid cells is associated with improved survival in patients with colorectal carcinoma. *Int J Cancer* 2011; 128(11):2663-72.
24. Donovan LK, Delaidelli A, Joseph SK, et al. Locoregional delivery of CAR T cells to the cerebrospinal fluid for treatment of metastatic medulloblastoma and ependymoma. *Nat Med* 2020;doi:10.1038/s41591-020-0827-2.
25. Theruvath J, Sotillo E, Mount CW, et al. Locoregionally administered B7-H3-targeted CAR T cells for treatment of atypical teratoid/rhabdoid tumors. *Nat Med* 2020;doi:10.1038/s41591-020-0821-8.
26. Choi BD, Suryadevara CM, Gedeon PC, et al. Intracerebral delivery of a third generation EGFRvIII-specific chimeric antigen receptor is efficacious against human glioma. *J Clin Neurosci* 2014;21(1):189-90.
27. Brown CE, Badie B, Barish ME et al. Bioactivity and Safety of IL13R α 2-Redirected Chimeric Antigen Receptor CD8⁺ T Cells in Patients with Recurrent Glioblastoma. *Clin Cancer Res* 2015;21(18):4062-72.
28. Baron JL, Madri JA, Ruddle NH, et al. Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. *J Exp Med* 1993;177:57-68.
29. Zhu X, Fallert-Junecko BA, Fujitaet M et al. Poly-ICLC promotes the infiltration of effector T cells into intracranial gliomas via induction of CXCL10 in IFN α and IFN γ dependent manners. *Cancer Immunol Immunother* 2010;59:1401-9.
30. Liffers K, Lamszus K, Schulte A. EGFR Amplification, and Glioblastoma Stem-Like Cells. *Stem Cells Int* 2015;427518.
31. Martinelli E, De Palma R, Orditura M, et al. Anti-epidermal growth factor receptor monoclonal antibodies in cancer therapy. *Clin Exp Immunol* 2009;158(1):1-9.
32. Alter G, Malenfant JM, Altfeld M. CD107a as a functional marker for the identification of natural killer cell activity. *J Immunol Methods* 2004;294(1-2):15-22.
33. Radoja S, Saio M, Schaer D, et al. CD8 (+) tumor-infiltrating T cells are deficient in perforin-mediated cytolytic activity due to defective microtubule-organizing center mobilization and lytic granule exocytosis *J Immunol* 2001;167(9):5042-51.
34. Sharifi Z, Abdulkarim B, Meehan B, et al. Mechanisms and Antitumor Activity of a Binary EGFR/ DNA-Targeting Strategy Overcomes Resistance of Glioblastoma Stem Cells to Temozolomide. *Clin Cancer Res* 2019;25(24):7594-608.

RESEARCH ARTICLE

THE IMPACT OF COVID-19 ON NEW CANCER DIAGNOSES

L. Mangone ¹, P. Giorgi Rossi ¹, I. Bisceglia ¹, R. Grilli ², C. Pinto ³

¹ Epidemiology Unit, Azienda Unità Sanitaria Locale, IRCCS di Reggio Emilia, Reggio Emilia, Italy

² Clinical Governance Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

³ Medical Oncology, Clinical Cancer Centre, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

CORRESPONDING AUTHOR:

Lucia Mangone
Epidemiology Unit
Azienda Unità Sanitaria Locale
IRCCS di Reggio Emilia
via Amendola 2
42122 Reggio Emilia, Italy
E-mail: mangone.lucia@ausl.re.it
ORCID: 0000 0003 4850 2678

Doi: 10.48286/aor.2021.05

ABSTRACT

Recent studies have assessed the impact of the COVID-19 pandemic and related control measures on the number of new cancer diagnoses. The aim of this work was to evaluate the impact of the lockdown on new cancer diagnoses.

To compare the incidence of tumors in 2020 with that in 2019, we used the data from the pathology anatomy reports available until the 31st of August 2020 and collected by the Reggio Emilia Cancer Registry. Over 90% of all incident cancer cases have microscopic confirmation. We reported the variations (number of cases and % values) of all tumors and of the main sites by sex, age and period.

From the 1st of January to the 31st August 2020, we recorded 3,548 new cancer diagnoses, 14% fewer than in the corresponding months of 2019. For all cancers, the pre-lockdown period (January-February) had a similar number compared to the same months in 2019; the lockdown period (March-May)

showed a decrease (-35%), but the post-lockdown (June-August) period showed similar numbers to those observed in 2019 (-2%). The difference is more evident in males and in the elderly. Breast cancer shows an increase in the first months (24%), a decrease during the lockdown (-35%), but a rapid recovery of the diagnosis after the lockdown (11%). Lung cancer showed a decrease in incidence in all three periods (-18%, -22%, and 21%, respectively). Colorectal cancers shows similar value during the first two months (-4%), a large decrease during lockdown (-53%), but an immediate return to normality after lockdown (-4%). Prostate cancer declined sharply during lockdown (-32%), as well as haematological cancers (-49%).

We observed a sharp decrease in cancers diagnosed during lockdown compared to the same period in 2019 particularly evident for the two cancers of organized screening programs (breast and colorectal cancer) and in the older people.

KEY WORDS

Covid-19; cancer; new diagnoses; period; age; screening.

INTRODUCTION

The SARS-CoV-2 virus first appeared in Italy at the end of January 2020, with an outbreak of infections detected in Codogno, Lombardy, after the first diagnosis of a COVID-19 in Italy case without any link to travel exposure on the 21st February 2020. The initial 16 confirmed cases increased to 60 the following day, with the first deaths reported in those same days (1). The Prime Minister Decree of the 9th of March 2020 (*I stay at home*) closed all non-essential businesses; this decree was in force until 16 May, when restrictions were gradually lifted; the ban on inter-regional travel was lifted on the 3rd of June. The three-month lockdown saw a slowdown in many diagnostic activities and a stop to the three organized screening programs in Italy, *i.e.*, cervical, colorectal, and breast cancer screening.

The aim of this work was to evaluate the impact that the COVID-19 pandemic and the resulting containment measures have had, in quantitative terms, on new cancer diagnoses.

The study was conducted in the Province of Reggio Emilia, a province in Northern Italy characterized both by a high incidence of tumors and by a high incidence of SARS-CoV-2 infections (2).

METHODS

The study presents preliminary data from the Reggio Emilia Cancer Registry, whose primary task is to monitor temporal and spatial variations in cancer incidence. The Reggio Emilia Cancer Registry (CR) has been approved by the provincial Ethics Committee of Reggio Emilia (Protocol n. 2014/0019740 of 04/08/2014).

Data source

The Reggio Emilia CR registers all new cancer diagnoses occurring in people residing in the Reggio Emilia province. The main information sources of the CR are the anatomic pathology reports, the hospital discharge records, and mortality data. The CR has been active since 2000 and has registered all incident cases since 1996 (3).

To compare cancer incidence in 2020 with that in 2019, we used data from the most complete among the cancer registry data sources, the only one that was already complete up to end of August 2020, *i.e.*, the anatomic pathology reports from the only Local Histopathology and Cytopathology Network active in the province. Cancer Registry operators conducted the initial case assessment for eligibility for incidence (excluding prevalent cases, non-residents, and non-invasive cancer diagnoses) according to international registration rules (4).

Analyses

We analyzed all tumor sites and the principal sites: breast, prostate, colorectal, lung, and haematologic cancers. For all sites we also report analyses by sex and age group (0-64, 65-79, 80+).

We first compared absolute numbers of new diagnoses per month occurring in 2019 and in 2020 for the period January-August. We also present the percentage change of 2020 compared to 2019 per month.

Data are also grouped as follows: pre-lockdown (January-February), full lockdown (March-May), and post-lockdown (June-August). We also report percentage differences between 2020 and 2019 in the three periods, with relative 95% confidence intervals (95%CI) estimated on Poisson distribution assuming equal denominator.

The study has an estimated power to identify a one third reduction in incidence comparing the three month period of the lockdown in 2020 with 2019 of 90% with alpha 5% for breast cancer, and approximately 70% for lung, colorectal and prostate cancer. Therefore we did not explore changes in incidence for cancer sites or subgroups with incidence lower than that of colorectal cancer, *i.e.*, about yearly crude incidence rate of cyto/histologically confirmed cases of about 60/100,000 inhabitants.

RESULTS

From the 1st of January to the 31st of August 2020, 3,548 new cancer diagnoses in the province of

MONTH	ALL SITES		BREAST		LUNG		COLORECTAL		PROSTATE		HAEMATOLOGIC	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
January	664	637	95	108	32	27	39	36	29	41	34	41
February	560	589	54	77	34	27	32	32	36	27	25	35
PRE-LOCKDOWN	1224	1226	149	185	66	54	71	68	65	68	59	76
March	564	382	52	39	26	35	45	20	28	30	33	17
April	458	293	42	27	26	18	21	5	29	16	32	23
May	558	346	59	33	38	17	27	19	34	16	45	16
LOCKDOWN	1580	1021	153	99	90	70	93	44	91	62	110	56
June	441	470	44	42	29	22	20	22	16	18	28	38
July	497	502	50	52	26	25	20	18	33	17	33	43
August	386	329	31	45	26	17	17	15	17	14	24	21
POST-LOCKDOWN	1324	1301	125	139	81	64	57	55	66	49	85	102
TOTAL	4128	3548	427	423	237	188	221	167	222	179	254	234

Table I. Number of new cancer diagnoses reported by the Reggio Emilia pathology network for all cancer sites and for the most frequent cancer sites, by month and period, 2019 and 2020, Reggio Emilia, Italy.

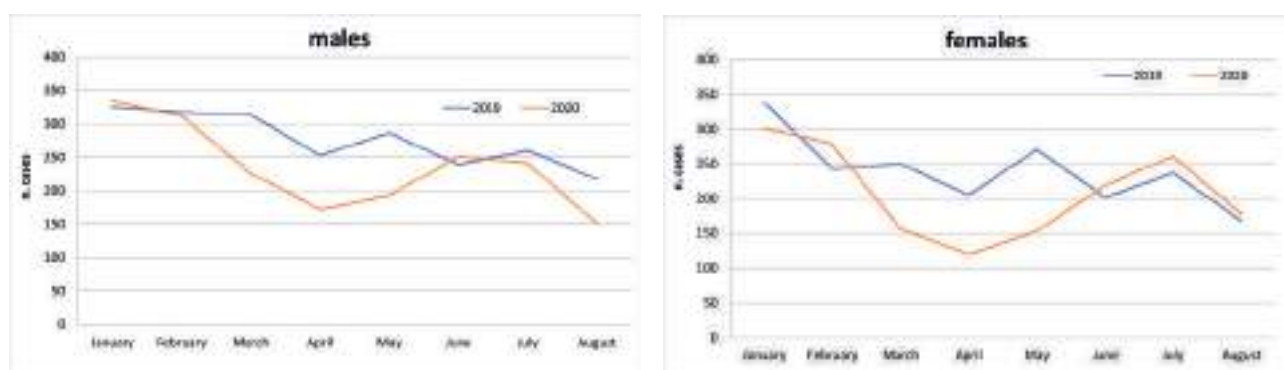


Figure 1. Number of cyto/histologically confirmed cases by month in 2020 and in 2019, for all sites by sex. Reggio Emilia, Italy.

Reggio Emilia were recovered from the pathology records, 14% less than in the corresponding months of 2019 (**table I**), more evident in males (- 15%) than in females (- 13%) (**figure 1**). Concerning age, the difference is minimal for the age group 0-64 years (- 8%), more marked for the age 65-74 (- 17%), and 80 + (- 17%) (**figure 2**).

Looking at specific cancer sites, 423 diagnoses of breast cancer were made (- 1%), 188 of lung cancer (- 21%), 167 of colorectal cancer (- 24%), 179 of prostate cancers (- 19%), and 234 haematologic cancers (- 8%) (**table I**).

Regarding the period trend in 2020 (**table II**), January and February (pre-lockdown) had a similar number of diagnoses for all cancers compared

to the same months in 2019 (0%, 95%CI - 8% to + 8%), while March, April, and May (full lockdown) showed a decrease in the number of diagnoses (- 35%, 95%CI - 40% to - 30%). However, June, July, and August (post-lockdown) showed numbers of diagnoses that were slightly lower than those observed in 2019 (- 14%, 95%CI - 20% to - 6%).

For breast cancer, there were more diagnoses during pre-lockdown than in the same period in 2019 (+ 24%, 95%CI 0% to + 55%). Incidence, however, dropped during lockdown (- 35%, 95%CI - 50% to - 16%) but increased slightly in the post-lockdown period (+ 11%, 95%CI - 13% to + 43%).

Lung cancer showed a decline in incidence throughout lockdown (- 22%, 95%CI - 44% to + 7%),

	PRE LOCKDOWN	95% CI	LOCKDOWN	95% CI	POST LOCKDOWN	95% CI
All sites	0%	- 8% to + 8%	- 35%	- 40% to - 30%	- 14%	- 20% to - 6%
Colorectal	- 4%	- 32% to + 35%	- 53%	- 68% to - 32%	- 4%	- 35% to + 42%
Lung	- 18%	- 44% to + 19%	- 22%	- 44% to + 7%	- 21%	- 44% to + 11%
Breast	24%	0% to + 55%	- 35%	- 50% to - 16%	11%	- 13% to + 43%
Prostate	5%	- 27% to + 49%	- 32%	- 51% to - 5%	- 26%	- 50% to + 9%
Haematologic	29%	- 10% to + 84%	- 49%	- 64% to - 29%	20%	- 11% to + 62%

Table II. Percentage change for the three periods, pre-lockdown (January, February), lockdown (March, April, May), post-lockdown (June, July, August) in 2020 compared to the same periods in 2019, for all cancers and by cancer site, Reggio Emilia, Italy.

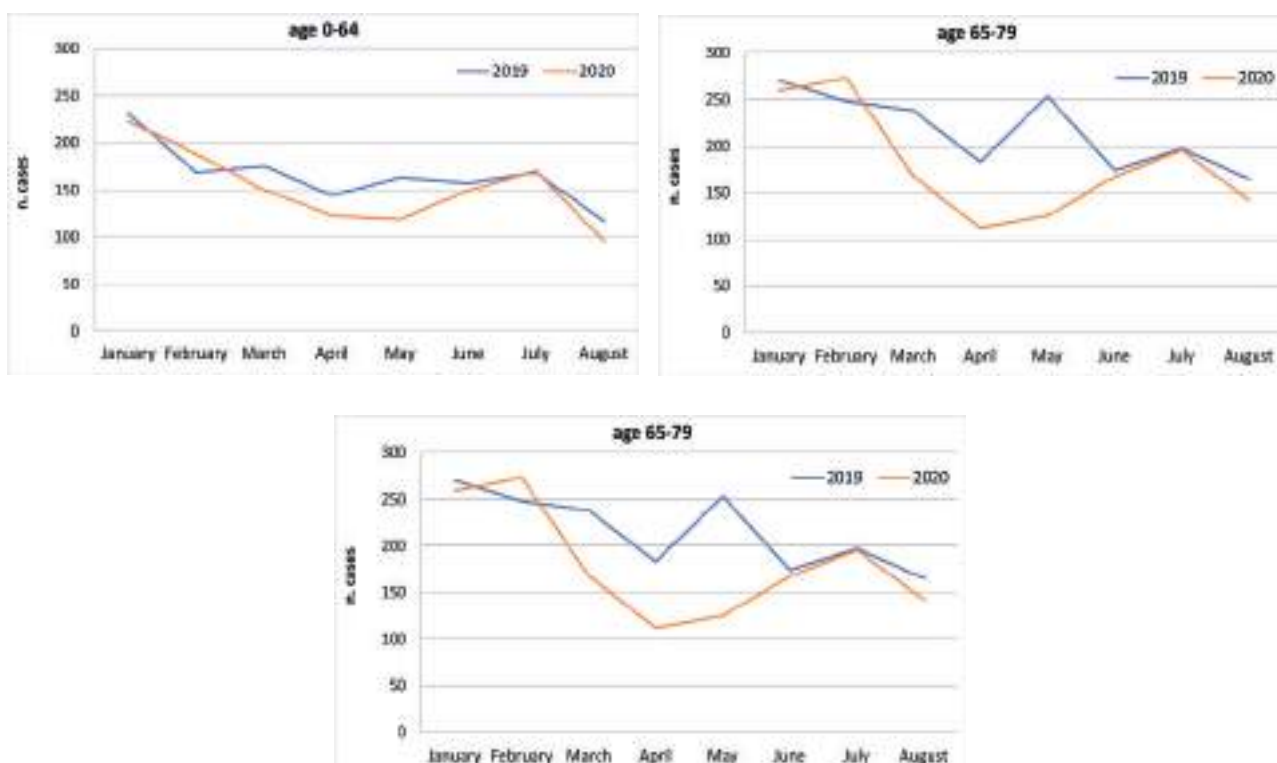


Figure 2. Number of cyto/histologically confirmed cases by month in 2020 and in 2019, for all sites by age group, Reggio Emilia, Italy.

but a decrease was also observed in pre-lockdown (- 18%, 95%CI - 44% to + 19%) and in post-lockdown (- 21%, 95%CI - 44% to + 11%).

The number of colorectal cancer diagnoses during pre-lockdown was similar to that of 2019 (-4%, 95%CI - 32% to + 35%); the large decrease in numbers seen during lockdown (- 53%, 95%CI - 68% to - 32%) immediately returned in post-lockdown to the numbers

observed in 2019 (- 4%; 95%CI - 35% to + 42%).

There was a sharp decline in prostate cancer diagnoses during lockdown (- 32%, 95%CI - 51% to - 5%), which persisted through post-lockdown (- 26%, 95%CI - 50% to + 9%). Haematologic cancer diagnoses had higher numbers both in the pre- and post-lockdown periods than in 2019, (+ 29%, 95%CI - 10% to + 84% and + 20%, 95%CI - 11% to

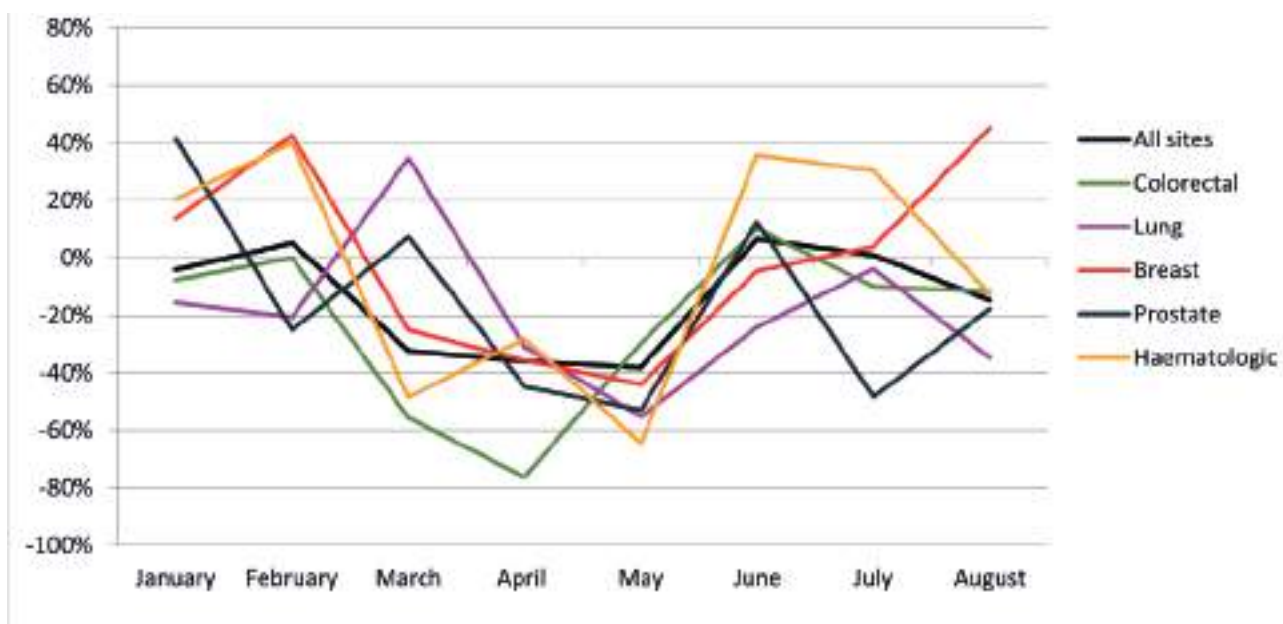


Figure 3. Percentage change for each month in 2020 compared to the same month in 2019, for all cancers and by cancer site: 2020 vs 2019, Reggio Emilia, Italy.

+ 62%, respectively), but registered a notable decrease during lockdown (- 49%, 95%CI - 64% to - 29%). For all tumor sites and for individual sites, percentage changes are shown in **figure 3**.

DISCUSSION

We observed a sharp decrease in the number of cancers diagnosed in Italy during the March-May 2020 lockdown in contrast to the spread of pandemic, compared to the same period in 2019. While new diagnoses of cancer increased after lockdown, the number remained slightly lower than in the previous year. The decrease was stronger in over 65 and in females. This trend is appreciable for all cancer sites analyzed, except for lung cancer, where an anomalous peak in diagnoses in March 2020 partially compensated for the drop observed during the following two months of full lockdown. Instead, the drop was particularly evident for the two cancers that are the target of organized screening programs, *i.e.*, breast and colorectal cancers, and for haematologic cancers. Recent studies indicate how much the COVID-19 pandemic has impacted the management of patients with cancer and how the treatment modality has also changed (5-7). Certainly, balancing the harms and benefits of exposing patients at risk of

infection and of a worse prognosis due to diagnostic delay has been a daily challenge in these last months (8). The decrease in diagnoses during lockdown was larger for people over 65 and this is consistent with decision making based on the balance of risk of SARS-CoV-2 infection and the benefit of timely diagnosis. In fact, the role of age as risk factor for SARS-CoV-2 infection and COVID-19 severity was clear since the very beginning of pandemic spread in western countries, thus it is not surprising that older people renounced undergoing testing and assessment during lockdown, by their own decision or advised by their physicians.

Two major studies have evaluated the reduction in the number of new cancer diagnoses during the initial period of the COVID-19 pandemic. The first study (9) reported data from the US and the UK to compare the number of cancer diagnoses in January-April 2020 with the number in the same months of 2019. The number of new cases identified in January and February 2020 was slightly higher than that in January-February 2019 (+ 11.5% and + 4.3 %, respectively). Cancer cases in March and April 2020 versus March and April 2019 decreased by - 22.3% and - 65.2%, respectively. Identified patients with melanoma, prostate cancer, or breast cancer displayed the largest decrease, with - 51.8%, - 49.1%, and - 47.7% cases, respectively. The second major study, conducted in the Netherlands (10), also showed a decline in the

incidence of cancer, more marked in the age group 80+ years and for breast cancer.

Our study confirms that the situation in our province in January and February 2020 was comparable to that in the same period in 2019. This is consistent with the data from the UK: - 1.9% and + 4.4% in January and in February 2020, respectively (9). In the three months of lockdown we observed a more marked but also a more homogenous reduction in cancer diagnoses in our province (- 32%, - 36%, and - 38% in March, April, and May, respectively) compared to that in other countries: in the USA the reduction was - 10.9% and - 65.2% for the months of March and April, respectively, and in the UK it was - 10.9% and - 64.6% (9).

This greater homogeneity probably reflects the duration and the strictness of the lockdown in Italy, which has been recognized as one of the most restrictive and long lasting among the Western world. An interesting feature of our study is that we were able to also examine what happened after the end of lockdown. As soon as lockdown ended, there was a rapid resumption of new diagnoses of cancer (+ 7% in June, + 1% in July). In August 2020, in addition to the usual decrease in diagnoses during this summer month (also observed in 2019), there was a further decrease of 15%. This may have been due to the backlog of vacation time taken by those health workers who could not take time off during the initial phase of the COVID-19 emergency but also to the fact that the patients themselves may have preferred to postpone surgery for a few weeks.

Our study confirms a decline in diagnoses of prostate, breast, and haematologic cancers and an even more considerable decline in colon cancer diagnosis (- 53%), certainly due to the strong impact of the suspension of the organized screening program. It is worth noting that the number of diagnoses of colorectal and breast cancer in the post-lockdown period reached the same numbers observed in 2019, once the screening programs resumed, despite slightly fewer monthly invitations compared to the pre-COVID-19 era. It will be interesting to see whether the temporary suspension

of the screening programs had any impact on the stage at diagnosis (11, 12).

A delay in tumor diagnosis is also indirectly documented by the lengthening of waiting times for a biopsy (13) and by the delay in receiving histopathological or clinical reports (14). However, while this decrease in diagnoses was unavoidable in the earlier months of the pandemic, it is very important to resume diagnostic pathways as soon as possible; any further delay could result in a large number of excess deaths and of years of life lost (15, 16). This is especially true for those cancer sites that progress rapidly and for which no screening is available; timely diagnosis at symptom onset may make the difference for cancers such as lung and pancreas cancer. This study includes only the anatomy pathology reports, the most informative source in terms of the specificity of the diagnosis as well as the one with highest sensitivity, considering that over 95% (3) of all incident cancer cases in the Reggio Emilia CR has a microscopic confirmation. A limitation of our study is that the numbers are small because they refer to a small geographical area. Counterweighing this, however, is the fact that ours are population-based data covering an interval of 8 months, including the 3 months following lockdown, when economic and healthcare activities in Italy resumed. The complete suspension of some clinical activities during the early months of the COVID-19 emergency was the result of many hospital departments being converted into COVID-19 wards to cope with the high numbers of cases. However, while urgent oncological examinations and therapies were never suspended, there was still a drastic decline in new cancer diagnoses, particularly in older people. Indeed, general practitioners referred to the oncologist only those cases that could not be postponed. It is important now to resume diagnostic pathways to limit as much as possible the impact of diagnostic delay on the prognosis of these patients.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

1. Available at: www.salute.gov.it. Accessed 18/12 2020.
2. Pinto C, Berselli A, Mangone L et al. SARS-CoV-2 Positive Hospitalized Cancer Patients during the Italian Outbreak: The Cohort Study in Reggio Emilia Biology 2020;9(8):181.
3. Mangone L, Borciani E, Michiara M, Vicentini M, Carrozzi G, Mancuso P, Sacchetti C, Giorgi Rossi P. I tumori nelle province dell'Area Vasta Emilia Nord. Piacenza, Parma, Reggio Emilia e Modena. Anni 2013-2014.
4. Bray F, Parkin DM. Evaluation of data quality in the cancer registry: principles and methods. Part I: comparability, validity and timeliness. Eur J Cancer 2009;45:747-55.
5. Sheng JY, Santa-Maria CA, Mangini N, et al. Management of Breast Cancer During the COVID-19 Pandemic: A Stage- and Subtype-Specific Approach. JCO Oncol Pract 2020.
6. Akula SM, Abrams SL, Steelman LS, et al. Cancer therapy and treatments during COVID-19 era. Adv Biol Regul 2020;77:100739.
7. Papautsky EL, Hamlish T. Patient-reported treatment delays in breast cancer care during the COVID-19 pandemic. Breast Cancer Res Treat. 2020;9:1-6.
8. Košir U, Loades M, Wild J, Wiedemann M, et al. The impact of COVID-19 on the cancer care of adolescents and young adults and their well-being: Results from an online survey conducted in the early stages of the pandemic. Cancer 2020;22:10.
9. London JW, Fazio-Eynullayeva E, Palchuk MB, Sankey P, McNair C. Effects of the COVID-19 Pandemic on Cancer-Related Patient Encounters. JCO Clin Cancer Inform 2020;4:657-65.
10. Dinmohamed AG, Visser O, Verhoeven RHA, et al. Fewer cancer diagnoses during the COVID-19 epidemic in the Netherlands. Lancet Oncol 2020;21(6):750-1.
11. Balzora S, Issaka RB, Anyane-Yeboah A, Gray DM 2nd, May FP. Impact of COVID-19 on colorectal cancer disparities and the way forward. Gastrointest Endosc 2020:20.
12. Del Vecchio Blanco G, Calabrese E, Biancone L, Monteleone G, Paoluzi OA. The impact of COVID-19 pandemic in the colorectal cancer prevention. Int J Colorectal Dis 2020:1-4.
13. Yang Y, Shen C, Hu C. Effect of COVID-19 Epidemic on Delay of Diagnosis and Treatment Path for Patients with Nasopharyngeal Carcinoma. Cancer Manag Res 2020;12:3859-64.
14. Zadnik V, Mihor A, Tomsic S, et al. Impact of COVID-19 on cancer diagnosis and management in Slovenia - preliminary results. Radiol Oncol 2020;29;54(3):329-34.
15. Maringe C, Spicer J, Morris M, Purushotham A, Nolte E, Sullivan R, et al. The impact of the COVID-19 pandemic on cancer deaths due to delays in diagnosis in England, UK: a national, population-based, modelling study. Lancet Oncol 2020;21(8):1023-34.
16. Sud A, Torr B, Jones ME, et al. Effect of delays in the 2 week-wait cancer referral pathway during the COVID-19 pandemic on cancer survival in the UK: a modelling study. Lancet Oncol 2020;21(8):1035-44.

RESEARCH ARTICLE

PREVENTION OF OXALIPLATIN-INDUCED PERIPHERAL NEUROPATHY IN COLORECTAL CANCER: THE PLANET TRIAL EXPERIENCE

S. Delfanti ¹, R. Tancredi ¹, F. Serra ¹, T. Monaco ¹, C. Gandini ¹, A. Ferrari ¹,
C. Tinelli ², S. Brugnattelli ¹

¹ Medical Oncology Unit, IRCCS Policlinico San Matteo, Pavia, Italy

² Biometrics and Statistics Service, IRCCS Policlinico San Matteo, Pavia, Italy

CORRESPONDING AUTHOR:

Francesco Serra
Medical Oncology Unit
IRCCS Policlinico San Matteo
viale Golgi Camillo 19,
27100 Pavia, Italy
E-mail: francesco.serra03@universitadipavia.it
ORCID: 0000-0001-9784-2551

Doi: 10.48286/aor.2021.06

ABSTRACT

Oxaliplatin-induced peripheral neuropathy (OIPN) is a common side effect in patients receiving chemotherapy for colorectal cancer (CRC) and it remains the most frequent dose-limiting toxicity, affecting especially quality of life (QoL) of patients. The best known pathogenetic mechanisms is the production of reactive oxygen species (ROS) and their negative activity at the axonal level. Consequently, medical research focused on the hypothesis that antioxidant substances may be efficacious in preventing OIPN. The purpose of our work is to report the experience of the PLANET trial (Oxaliplatin Neurotoxicity Prevention Trial) and provide a constructive discussion on one of the oncological toxicities still today little controlled by medical therapies.

The PLANET trial was a monocentric, prospective, randomized, placebo-controlled, double blind, phase II clinical study designed to investigate if the association of vitamin E and super oxide dismutase (SOD) is able to prevent OIPN in colorectal cancer patients receiving oxaliplatin-based chemotherapy regimens. Primary endpoint was the assessment of OIPN incidence and severity in the two treat-

ment arms. Secondary endpoints were the correlation between the OIPN and the oxaliplatin dose in terms of symptoms intensity, type of neuropathy and quality of life; finally the OIPN duration along the follow-up checks. 47 patients with CRC, operated or in advance stage, candidates for oxaliplatin-based chemotherapy, were enrolled from January 2014 to October 2015 in our Center and randomized into the two groups, the experimental arm (24 patients) and the placebo arm (23 patients). The study included an analysis of at least 18 months, 6 of treatment and 12 of follow-up. In the global cohort analyzed, 32.5% of patients reported development of paresthesias during chemotherapy treatment, with an increase of symptoms intensity in cycles progression of active treatment and a reduction during follow-up. After 12 months of follow-up, 50% of participants experienced complete relief of paresthesias while 50% had persistent symptoms. But the most important finding was the lack of statistical differences between the two arms in terms of neuropathy incidence, toxicity and variations in QoL.

OIPN represents a still poorly understood oncological toxicity. In addition to oxidative stress, there are other pathogenetic mechanisms, partly clear and partly unknown. Scientific research is trying to better study them and to develop efficacious treatment strategies against this toxicity. Over the years there have been many attempts to use various drugs but with unsatisfactory results. Our PLANET experience leads us to conclude that the association of vitamin E and SOD has not proved efficacious in preventing the OIPN. The main reasons are due to the small-

ness of the sample analyzed and the pathogenetic complexity of the phenomenon in which oxidative stress represents, according to medical literature, only a part of the mechanisms responsible for the OIPN. The positive note is that the treatment has a good tolerance. OIPN remains an open chapter of oncology for which more information is needed in order to identify an efficacious treatment strategy. The prevention of this toxicity would allow a better management of platinum-based chemotherapy and an improvement in the quality of life of patients.

KEY WORDS

Oxaliplatin-induced peripheral neuropathy; colorectal cancer; vitamin E; super oxide dismutase.

IMPACT STATEMENT

With our work, mainly addressed to oncologist colleagues dealing with gastrointestinal malignancies, we wish to provide our monocentric experience on one of the still unresolved chemotherapy toxicities.

INTRODUCTION

Colorectal cancer (CRC) represents the third most frequent oncological disease in men and women. Despite this high incidence, the mortality is low with a greater than 60% 5-years overall survival.

Over the few last decades there is an increase in survival, related to early diagnosis and treatment improvements (1). One of the most widely used treatment regimens, in both early and advanced patients, is oxaliplatin-based chemotherapy. Oxaliplatin is a third-generation platinum compound with a significant antineoplastic activity. It forms an essential part of colorectal cancer neoadjuvant, adjuvant and even in palliative chemotherapy regimens, particularly in combination with 5-FU and leucovorin in FOLFOX or in XELOX regimens. Its mechanism of action involves the cross-linking with the strands of DNA, inhibiting DNA replication and transcription (2-4). Like all drugs, especially oncological ones, oxaliplatin also has side effects. The main one is the peripheral neuropathy (5).

Oxaliplatin-Induced Peripheral Neuropathy (OIPN) occurs in two distinct forms. Firstly, it appears as an acute cold-triggered sensory neuropathy which affects 85-90% of patients and which develops shortly after infusion of the drug with symptoms like paresthesias and/or disesthesias in the distal extremities, in the perioral region and rarely in the

throat. Symptoms reach their peak three days after administration, then tend to subside over the next 7 days. The second form of OIPN is a chronic neuropathy which affect 10-15% of patients and which is correlated with the cumulative dose of oxaliplatin administered; it involves sensory loss, sensory ataxia and changes in proprioception. Common symptoms include numbness, tingling and/or burning pain. This type of neurotoxicity persists throughout treatment and increases in intensity with cumulative dose; symptoms resolve within 6-12 months of cessation of therapy, but in a small group of patients, symptoms persist for more than one year (6-8).

This oncological toxicity, both in the acute and in the chronic form, interferes with the patient's daily activities, negatively affecting his quality of life (9, 10). Regarding the pathogenetic mechanisms of OIPN, the best known is certainly the oxidative stress and the consequent axonal damage (11). Medical research has therefore focused on the hypothesis that antioxidant substances may be efficacious in preventing this problem (12).

The purpose of our work is to report the experience of the PLANET trial (Oxaliplatin Neurotoxicity Prevention Trial) and provide a constructive discussion on one of the oncological toxicities still today little controlled by medical therapies.

MATERIALS AND METHODS

Study design, endpoints and evaluation systems

The PLANET trial was a monocentric, prospective, randomized, placebo-controlled, double-blind, phase II clinical study which evaluated the efficacy of a combination of vitamin E in the form of tocotrienols (Tocomax™) and super oxide dismutase (SOD) in preventing peripheral neuropathy in colorectal cancer patients. The study therefore had two arms: the experimental one consisting of the pharmacological combination and the control one based on placebo.

The primary endpoint was the assessment of OIPN incidence and severity between the two treatment arms. The secondary endpoints were the correlation between the OIPN and the oxaliplatin dose in terms of symptoms intensity, type of neuropathy and quality of life; finally, the OIPN duration along the follow-up checks.

The primary endpoint was assessed through the functional medical evaluation which attested the appearance of OIPN and its preliminary gradation according to the NCI-CTCAE (National Cancer Institute - Common Toxicity Criteria for Adverse Events). Oncologist assigned a score as follows: 0 = normal; 1 = weakness on physical exam and/or loss of reflexes or paresthesias not interfering with daily function; 2 = weakness and sensory alterations interfering with daily function; 3 = weakness and sensory alterations interfering with activities of daily living or requiring bracing or assistive devices; 4 = life threatening, paralysis, disabling (13). For the assessment of the secondary endpoints, the patient questionnaires were added to the functional medical evaluation. These were completed at baseline, prior to the start of treatment, at 3 and 6 months and every three months after treatment cessation during the 12 months of follow-up.

We selected two questionnaires validated and promoted by the EORTC (European Organization for Research and Treatment of Cancer). The first was the QLQ-CIPN20 (Quality of Life Questionnaire - Chemotherapy Induced Peripheral Neuropathy) which evaluated the link between the cumulative exposure to oxaliplatin and each aspect of the OIPN such as the type of neuropathy, the intensity of symptoms and the implications on quality of life. This tool included 20 items measurable through a

Likert scale ranging from 1 (not at all) to 4 (very much); scores were linearly transformed to 0-100 scale (14).

The second questionnaire was the QLQ-C30 (Quality of Life Questionnaire - Cancer) which assessed the quality of life of patients in relation to a large spectrum of physical, psychological and cognitive symptoms, not necessarily due to OIPN; each item was scored from 1 (not at all) to 4 (very much), except for the global QoL perception which ranged from 1 (very poor) to 7 (excellent). Similarly, to the first, also in this questionnaire the scores obtained were transformed into a 0-100 scale (15).

Finally, the same tools were used to define the OIPN duration after 3, 6, 9, 12, 15 and 18 months from the start of treatment.

Pharmacological rationale

In our experience we decided to use the combination of vitamin E and superoxide dismutase. These are two antioxidant substances that play a central role in fighting free radicals. Vitamin E is a powerful antioxidant which neutralizes reactive oxygen species (ROS) and actively protects cells from oxidative stress and in particular from lipid peroxidation. Superoxide dismutase, on the other hand, is an endogenous enzyme present in most human tissues which converts ROS into hydrogen peroxide, thus contributing to the reduction of oxidative stress.

We chose to use this drug combination for two main reasons: first of all, the best known and studied pathological mechanism of oxaliplatin-induced neuropathy is just the peripheral axonal damage due to oxidative stress. Secondly, we exploited the positive results of some preliminary studies which demonstrated the efficacy in the use of these molecules to prevent oxaliplatin neuropathy (12).

Patients population, analysis time and treatment plan

Colorectal cancer patients aged 18 years or more, with a good performance status (ECOG 0,1) were assigned to adjuvant or palliative chemotherapy with oxaliplatin after obtaining informed consent. Preexisting or actual neuropathy from any other cause - mellitus diabetes, chronic alcoholism, malnutrition or vitamin B deficiency, prior exposure to chemotherapy, pregnancy, and gluten intolerance were exclusion criteria. Forty-seven patients were enrolled from January 2014 to October 2015 in our Center, from a total of 80 expected patients. Due to a slowdown in enrollment, the study was closed ear-

ly. The diagram in **figure 1** illustrates patients' flow-chart. Baseline characteristics were equivalent for the two treatment arms and are reported in **table I**. Thirty-eight patients were operated and received adjuvant chemotherapy while nine patients were in advanced stage and received palliative chemotherapy. Of the total 47 patients, 24 were assigned to the experimental arm and 23 to the control arm.

The study included 18 months of analysis with a minimum of 6 months of active therapy and 12 months of follow-up. Treatment interruption before 6 months, because of toxicity or early chemotherapy discontinuation, was considered in the statistical analysis.

The drug combination was registered as Reclex® but, for the purpose of our study, it was made clearly unrecognizable. Reclex® retard enteric-coated controlled-release pills were administered daily during the whole chemotherapy treatment period (6 months). Even if one or more cycles of oxaliplatin were not administered the antioxidant drug was maintained. Drug administration was interrupted only in cases of severe oxaliplatin side effects. Patients were dropped from the trial if treatment was interrupted for more than seven days.

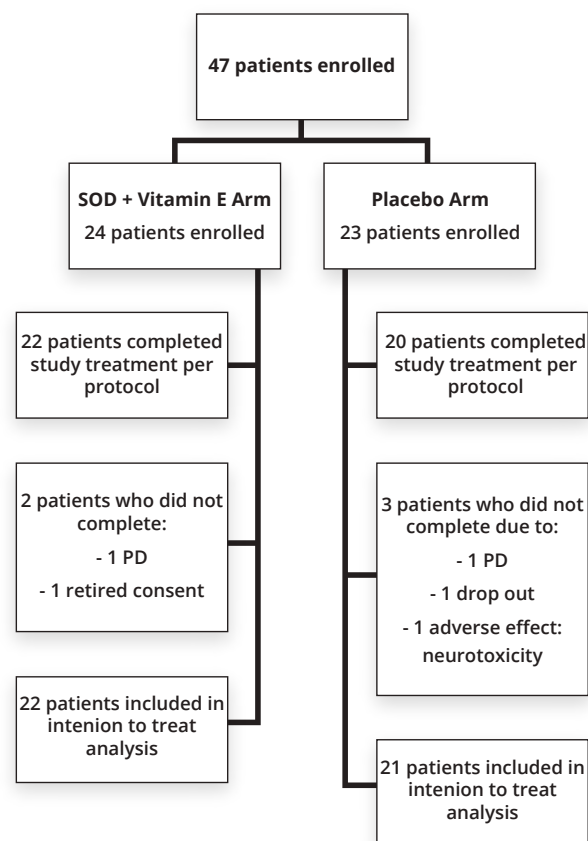


Figure 1. Patients flow-chart.

STATISTICAL ANALYSIS

All patients were recruited, treated and followed-up at the Medical Oncology Unit of IRCCS San Matteo Hospital of Pavia, in Italy. Patients were randomly assigned to Reclex® or placebo groups. The sample size required (40 patients for each group) was calculated from the hypothesized difference of 40% (control group) versus 10% (Reclex® group) in the neurotoxicity rate when power is set at 80%. Calculations used two-sided t tests with alpha error set at 0.05. Expecting a 10% of drop out, a total of 80 patients were considered for recruitment. Efficacy assessment is primarily conducted on an "intention-to-treat" approach. All randomized patients have been included in the data analysis: in cases of drop out and interrupted follow-up, patients were considered as not having achieved endpoint. Frequencies and percentages were calculated for qualitative data and comparisons were analyzed using chi-square test or Fisher exact test, as appropriate. Mean and standard deviation were used for describe quantitative variables, if normally distributed, otherwise median and interquartile range (IQR) were used. A t test for independ-

ent data (or equivalent non parametric test) was used to compare quantitative variables between two group. Linear regression models for repeated measures were used to analysis the changes over time of EORTC-CIPN20 and QLQ-C30 scores. Data were express as monthly change means with theirs Standard Errors (SE). A value of $p < 0.05$ was considered statistically significant. All tests were two-sided. The data analysis was performed using the STATA statistical package (version 15.0, 2017, Stata Corporation, College Station, Texas, USA).

RESULTS

Of the total 47 patients, 43 were analyzed as intention to treat population. Regarding the primary endpoint of the study, the incidence rates and grades of neurotoxicity were assessed during treatment, at the end of treatment and after 12 months of follow-up. During chemotherapy, 14 patients (32,5% of total) reported development of paresthesias. Incidence of NCI-CTCAE grades is described in **figure 2**. We observed an increase of

	RECLEX® RETARD (NO 24)	PLACEBO (NO 23)	ALL	P
Median Age (IQR)	59.0 (53.1 – 65.5)	64.6 (60.9 – 68.4)	62	0.0370
Female (%)	6 (25.0)	5 (21.7)	11	0.792
STAGE				
C1/C2 D	18 (47) 6 (67)	20 (53) 3 (33)	38 9	0.461
PLANNED CHEMOTHERAPY REGIMEN (%)				
XELOX FOLFOX4 FOLFOX6 (± bevacizumab or panitumumab)	4 (16.7) 14 (58.3) 6 (25.0)	9 (39.1) 11 (47.8) 3 (13.0)	13 25 9	0.211
COMPLETED TREATMENT				
XELOX FOLFOX4 FOLFOX6 (± bevacizumab or panitumumab)	3 (12.5) 14 (58.3) 5 (20.8)	9 (39.1) 9 (39.1) 2 (8.7)	12 23 7	0.075

Table I. Baseline patient characteristics.

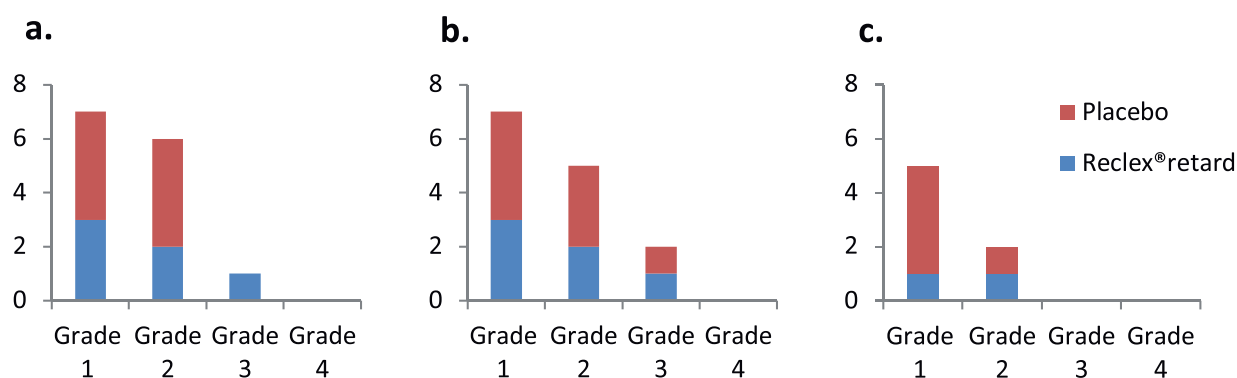


Figure 2. **a.** Distribution of NCI-CTCAE grading of paresthesia at appearance; **b.** at the end of chemotherapy; **c.** after 12 months follow-up.

symptoms intensity during active treatment and a reduction during follow-up (**figure 3**).

For the secondary endpoints, the QLQ-CIPN20 questionnaire showed no significant difference between treatment arms in terms of OIPN incidence, evolution, type of neuropathy, symptoms intensity and quality of life (**figure 4**). The average monthly change of the QLQ-C30 scores is reported in **table II**. Similarly, there were no differences between the two arms in terms of non-neurological toxicities evaluated using the second questionnaire (**figure 5**). Reported side effects can be attributed to known toxicity associated with chemotherapy and not to experimental compound or placebo. Therefore, SOD/tocotrienols association with chemotherapy was well tolerated.

After 12 months of follow-up, 50% of participants experienced complete relief of paresthesias, while 50% had persistent symptoms, in most cases with grade 1, although 2 patients still had grade 2. Our results partially reflect literature data on the incidence of paresthesias. The trial showed neurotoxicity in 32,5% of patients, a percentage slightly lower compared to the values present in literature (40-50% as mean value). However, grade 3 incidence is similar (9,3% in PLANET trial vs 10-20% in literature). In the literature, symptoms are reported to be reversible within 6-12 months after treatment conclusion. Similarly, in the PLANET trial, relief occurred in 6-12 months with a median of 4 months (6-8).

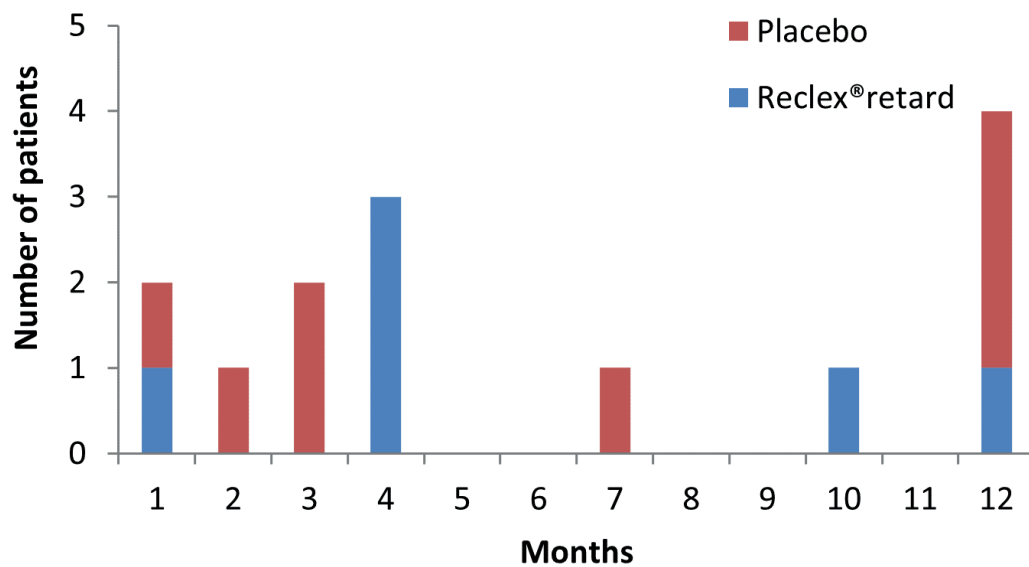


Figure 3. Duration of paresthesias; in majority of cases paresthesias resolved within 4 months. Some patients still have experience of symptoms after 7, 10 months or after termination of follow-up period (12 months).

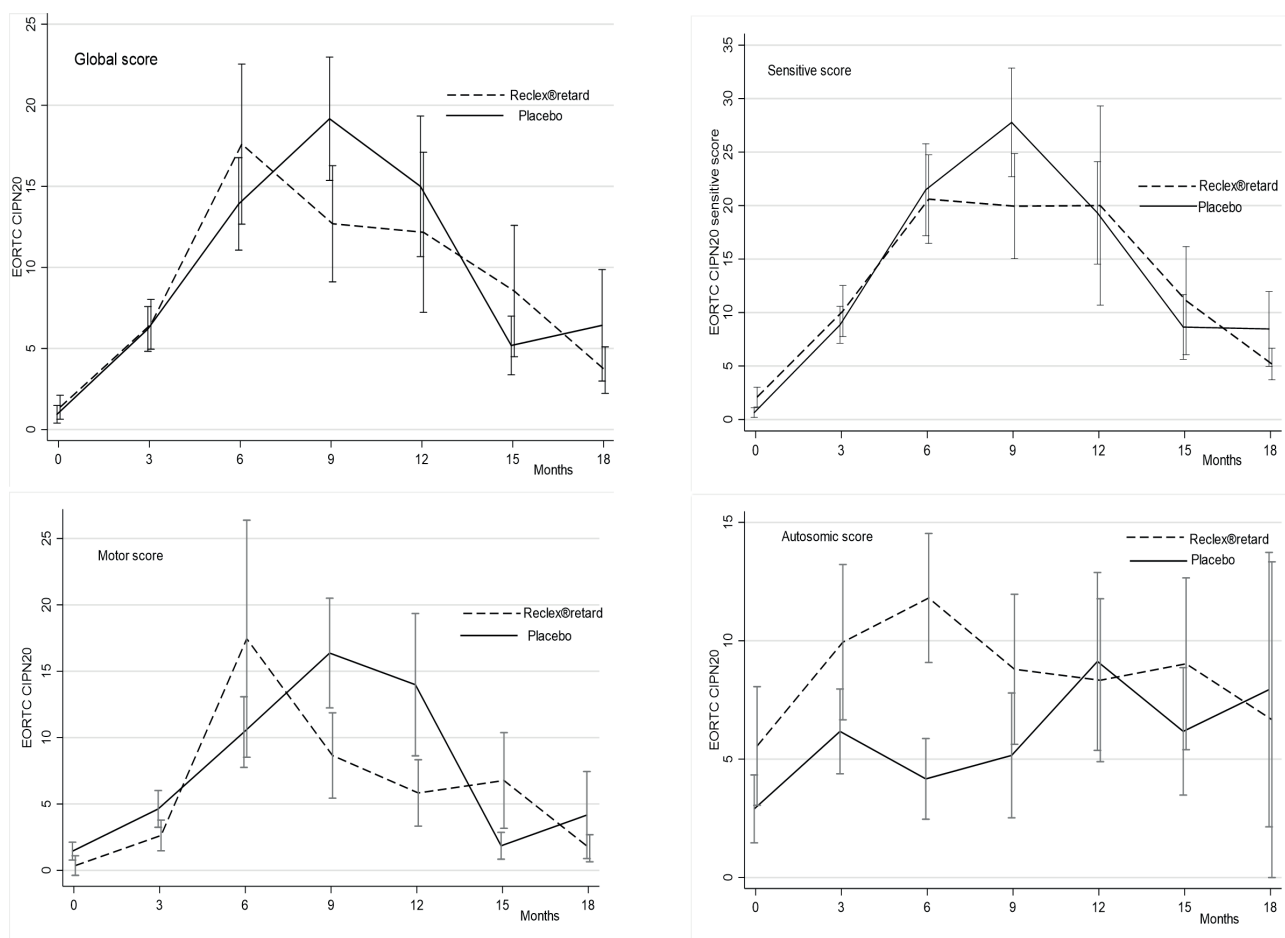


Figure 4. Results of QLQ-CIPN20.

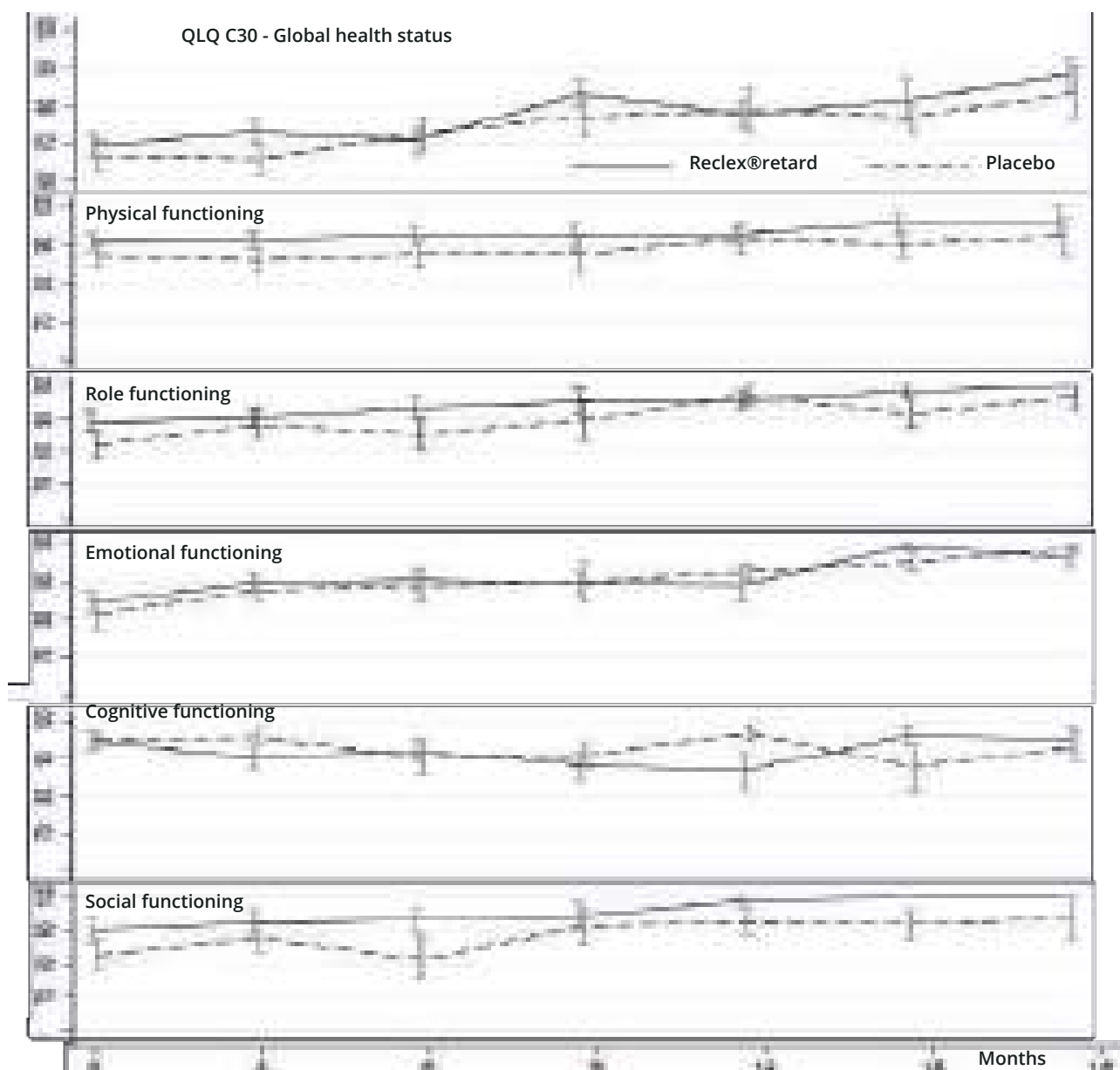


Figure 5. Results of QLQ-C30.

DISCUSSION

The exact mechanisms underlying the OIPN are unclear. It has been proposed that the acute form is a result of increased excitability of peripheral neurons caused by functional impairment of currents through Na^+ voltage-gated ion channels in nerve membranes after the chelation of calcium by oxalate, a metabolite of oxaliplatin. Chronic neuropathy results instead from the accumulation of platinum in dorsal root ganglion cells. A well-known pathological mechanism at the base of OIPN is the production of free radicals; these affect the integrity of the axonal membranes altering the correct conduction of nerve impulses (5-8, 11).

In the literature there is much evidence that several classes of chemotherapy agents, including Oxaliplatin, can lead to the formation of ROS (16). This is the reason why medical research promoted the use of antioxidants in the prevention of OIPN. The most varied molecules with antioxidant activity were tested, the main ones being vitamin E and glutathione. Overall, no specific molecule has been shown to be efficacious in preventing neurotoxicity; the best and most encouraging results were obtained with the use of glutathione (12, 17-20). The following are the most significant studies on the other substances most frequently used in the

	MEAN	SE	P
GLOBAL HEALTH STATUS	0.97	0.19	< 0.001
PHYSICAL FUNCTIONING	0.3	0.13	0.024
ROLE FUNCTIONING	0.71	0.14	< 0.001
EMOTIONAL FUNCTIONING	0.73	0.13	< 0.001
COGNITIVE FUNCTIONING	- 0.16	0.17	0.356
SOCIAL FUNCTIONING	0.66	0.17	< 0.001
FATIGUE	- 0.61	0.17	0.001
NAUSEA AND VOMITING	- 0.20	0.06	0.002
PAIN	- 0.38	0.15	0.012
DYSPNEA	0.02	0.16	0.879
INSOMNIA	- 0.53	0.19	0.008
APPETITE LOSS	- 0.34	0.14	0.022
CONSTIPATION	- 0.29	0.25	0.246
DIARRHEA	0.04	0.15	0.795
FINANCIAL DIFFICULTIES	- 0.26	0.25	0.303

Table II. Average monthly change of QLQ-C30.

management of oxaliplatin-induced neuropathy. Let's start from the Acetyl-L-carnitine; this is an enzyme which transports fatty acids into the mitochondria and appears to have an antioxidant action in the nervous system. Nevertheless, its efficacy in preventing OIPN has not been proven (21). Another substance put under the magnifying glass of scientific research is the Alpha-lipoic acid; it is an antioxidant molecule particularly active in the liver and nervous system. The only randomized study on its use concluded that it is unable to prevent OIPN and is also poorly tolerated (22).

A positive glimmer seemed to come from the intravenous administration of calcium and magnesium; several studies documented its efficacy without however obtaining a subsequent confirmation in the randomized and controlled clinical trials (23-30). The role of cannabinoids was studied in a small randomized trial which, however, was negative (31). Some researchers focused on cryotherapy and compression techniques, often combined, obtaining discordant results; in some trials it would seem

that the techniques have a positive result in the management of OIPN in others they do not (32-40). One substance which seemed promising is metformin which in a small randomized trial it shown to reduce the severity of oxaliplatin-induced neuropathy compared with the control arm. Clearly, more studies are needed to confirm its efficacy (41).

Many studies focused on the antiepileptic and antidepressant drugs. Gabapentin and pregabalin appear to produce positive effects on OIPN but without evidence confirmed by large-scale clinical studies (42-44). In the EFOF trial, Durand et al. demonstrated a clear efficacy of venlafaxine in preventing OIPN; in contrast, Zimmerman and colleagues failed to confirm any benefit using this drug (45, 46). The only drug in this class that has been shown to be efficacious in the management of chemotherapy-induced neuropathy is duloxetine. But its efficacy, as can be easily imagined, still needs to be validated (47-49). Afterwards the research focus shifted to molecules with neuroprotective activity. Manganofodipir is a chelate of manganese which seemed to reduce OIPN-related symptoms but its systematic use is not recommended due to the neurological toxicity resulting from the compound (50). Other neuroprotective molecules like amfostine, N3-polyunsaturated fatty acids, xaliproden, analgescine have been studied with promising effects in a few small trials (51-54).

Given the lack of real efficacy of global results, traditional oriental medicine has even been applied and in particular the role of herbs with a detoxifying action or the acupuncture. Again, the final results are negative (55-58).

Despite positive results obtained in several trials, there are no recommendations for any of these substances. Further prospective, randomized, controlled trials with larger sample sizes are needed to confirm these findings and to verify the clinical value of these agents in the management of OIPN. But most of all, what is now evident is that the pathogenesis of this problem is multifactorial, with partly still unknown mechanisms. Scientific research is trying to better study them and to develop efficacious treatment strategies against this toxicity.

The only useful approach that remains to oncologists is the management of oxaliplatin-based chemotherapy and more specifically the application of strategies such as dose reduction, slowing the infusion rate or the "stop and go" strategy. OP-TIMOX and CONcePT are two of the trials which have supported these OIPN prevention and treatment strategies (59-60).

The American Society of Clinical Oncology (ASCO) recently published the guidelines for the prevention and management of chemotherapy-induced peripheral neuropathy; it is a monumental review of the literature on the subject that rigorously analyzes nearly 40 substances, classifying them by strength of recommendation, evidence and clinical benefit. The innovative purpose of this work is the revision of randomized studies on the use of the analyzed substances in order to draw general and non-anecdotal conclusions. The ASCO expert panel confirms the appropriateness of clinical strategies for reducing or discontinuing chemotherapy and recognizes, with a moderate level of benefit, the treatment of OIPN with duloxetine reserving to obtain further data from subsequent and more targeted studies (61).

CONCLUSIONS

Our PLANET experience leads us to conclude that the association of vitamin E and SOD has not proved efficacious in preventing the OIPN. The main reasons are due to the smallness of the sample analyzed and the pathogenetic complexity of the phenomenon in which oxidative stress represents, according to medical literature, only a part of the mechanisms responsible for the OIPN. The positive note is that the treatment has a good tolerance.

As happens in most monocentric studies, a limit to be dealt with is just the small sample of patients studied which understandably affects the non-generalizability of the results obtained. Nevertheless, given the lack of solid data on this topic, we decided to publish our experience anyway, net of the final results. When we realized our work the ASCO guidelines had not yet been published so we based on preliminary studies which showed some positive result on the pharmacological activity and on the clinical benefit of the substances used. The

most important review we have today expresses a negative opinion on antioxidant substances, including vitamin E and superoxide dismutase, in the management of OIPN, which is consistent with our small experience (61).

A methodological limitation is represented by the use of questionnaires for patients to assess the intensity of neuropathy, which, however well compiled, remain absolutely subjective tools. The choice of this system, nevertheless, is due just to the fact that the symptoms of neuropathy, from paresthesia to pain, are subjective experiences for which there are no validated tools able to change the symptom in a clinical sign perfectly objective; to do this, it is necessary to use specialized tests, such as electroneuromyography, which on the whole are expensive, require specialized staff and do not represent ideal and flexible methods to be used frequently as in our study (62).

Finally, we can say that the antidote for this toxicity may not be a single drug but maybe a combination of several active molecules which reflects the multifactorial nature of problem.

OIPN remains an open chapter of oncology for which more information is needed in order to identify an efficacious treatment strategy. The prevention of this toxicity would allow a better management of platinum-based chemotherapy and an improvement in the quality of life of patients.

ACKNOWLEDGMENTS

We wish to thank the staff and all the patients who participated in this study.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

1. International Agency for Research on Cancer. Global cancer observatory 2018. Available at: <http://gco.iarc.fr/>. Accessed: 04/02/2021.
2. Raymond E, Chaney SG, Taamma A, Cvitkovic E. Oxaliplatin: a review of preclinical and clinical studies. *Ann Oncol* 1998; 9(10):1053-71.
3. Andre T, Boni C, Mounedji-Boudiaf L, et al. Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004; 350:2343-51.
4. De Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without Oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000; 18(16):2938-47.
5. Gamelin E, Gamelin L, Bossi L, Quasthoff S. Clinical aspects and molecular basis of oxaliplatin neurotoxicity: current management and development of preventive measures. *Semin Oncol* 2002; 29(5 Suppl 15):21-33.
6. Saif MW, Reardon J. Management of oxaliplatin-induced peripheral neuropathy. *Ther Clin Risk Manag* 2005;1(4):249-58.
7. Wilson RH, Lehky T, Thomas RR, Quinn MG, Floetter MK, and Grem JL. Acute oxaliplatin-induced peripheral nerve hyperexcitability. *J Clin Oncol* 2002;1(20):1767-74.
8. Bejers AJM, Mols F, Vreugdenhil G. A systematic review on chronic oxaliplatin-induced peripheral neuropathy and the relation with oxaliplatin administration. *Support Care Cancer* 2014;22(7):1999-2007.
9. Toftagen C, Donovan KA, Morgan MA, Shibata D, Yeh Y. Oxaliplatin-induced peripheral neuropathy's effects on health-related quality of life of colorectal cancer survivors. *Support Care Cancer* 2013;21(12):3307-13.
10. Mols F, Beijers T, Lemmens V, van der Hurk CJ, Vreugdenhil G, van de Poll-Franse LV. Chemotherapy-induced neuropathy and its association with quality of life among 2- to 11- year colorectal cancer survivors: results from the population-based PROFILES registry. *J Clin Oncol* 2013;31(21):2699-707.
11. Di Cesare Mannelli L, Zanardelli M, Failli P, Ghelardini C. Oxaliplatin-induced neuropathy: oxidative stress as pathological mechanism. Protective effect of silibinin. *J Pain* 2012;13(3):276-84.
12. Stankovic JSK, Selakovic D, Mihailovic V, Rosic G. Antioxidant Supplementation in the Treatment of Neurotoxicity Induced by Platinum-Based Chemotherapeutics. A Review. *Int J Mol Sci* 2020;21(20):7753.
13. National Cancer Institute, Department of Health and Human Services, Common Terminology Criteria for Adverse Events (Version 4.0; 2009). Available at: https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. Accessed: 04/02/2021.
14. European Organization for Research and Treatment on Cancer, Quality of Life Questionnaire, Chemotherapy Induced Peripheral Neuropathy 20. Available at: <https://www.eortc.org/app/uploads/sites/2/2018/08/Specimen-CIPN20-English.pdf>. Accessed: 04/02/2021.
15. European Organization for Research and Treatment on Cancer – Quality of Life Questionnaire, Cancer 30. Available at: <https://www.eortc.org/app/uploads/sites/2/2018/08/Specimen-QLQ-C30-English.pdf>. Accessed: 04/02/2021.
16. Conklin KA. Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness. *Integrative Cancer Therapies*. December 2004:294-300.
17. Argyriou AA, Chroni E, Koutras A, et al. Preventing paclitaxel-induced peripheral neuropathy: a phase II trial of vitamin E supplementation. *J Pain Symptom Manage* 2006;32(3):237-44.
18. Kottschade LA, Sloan JA, Mazurczak MA, et al. The use of vitamin E for the prevention of chemotherapy-induced peripheral neuropathy: results of a randomized phase III clinical trial. *Support Care Cancer* 2011; 19(11):1769-77.
19. Cascinu S, Catalano V, Cordella L, et al. Neuroprotective effect of reduced glutathione on oxaliplatin-based chemotherapy in advanced colorectal cancer: a randomized, double-blind, placebo-controlled trial. *J Clin Oncol*;20(16):3478-83.
20. Takimoto N, Sugawara S, Iida A, et al. Prevention of oxaliplatin-related neurotoxicity by glutathione infusions. *Gan To Kagaku Ryoho*;35(13):2373-6.
21. Hershman DL, Unger JM, Crew KD, et al. Randomized double-blind placebo-controlled trial of acetyl-L-carnitine for the prevention of taxane-induced neuropathy in women undergoing adjuvant breast cancer therapy. *J Clin Oncol* 2013;31(20):2627-33.
22. Guo Y, Jones D, Palmer JL, et al. Oral alpha-lipoic acid to prevent chemotherapy-induced periph-

- eral neuropathy: a randomized, double-blind, placebo-controlled trial. *Support Care Cancer* 2014;22(5):1223-31.
23. Gamelin L, Boisdron-Celle M, Delva R, et al. Prevention of oxaliplatin-related neurotoxicity by calcium and magnesium infusions: A retrospective study of 161 patients receiving oxaliplatin combined with 5-fluorouracil and leucovorin for advanced colorectal cancer. *Clinical Cancer Research* 2004;10(12 pt 1):4055-4061.
 24. Hochster HS, Grothey A, Childs BH: Use of calcium and magnesium salts to reduce oxaliplatin related neurotoxicity. *J Clin Oncol* 2007;25(25):4028-9.
 25. Gamelin L, Boisdron-Celle M, Morel A, et al. Oxaliplatin-related neurotoxicity: interest of calcium-magnesium infusion and no impact on its efficacy. *J Clin Oncol* 2008;26(7):1188-9; author reply 1189-90.
 26. Ishibashi K, Okada N, Miyazaki T, Sano M, Ishida H. Effect of calcium and magnesium on neurotoxicity and blood platinum concentrations in patients receiving mFOLFOX6 therapy: a prospective randomized study. *Int J Clin Oncol* 2010;15(1):82-7.
 27. Grothey A, Nikcevic DA, Sloan JA, et al. Intravenous calcium and magnesium for oxaliplatin-induced sensory neurotoxicity in adjuvant colon cancer: NCCTG N04C7. *J Clin Oncol* 2011;29(4):421-7.
 28. Knijn N, Tol J, Koopman M, et al. The effect of prophylactic calcium and magnesium infusions on the incidence of neurotoxicity and clinical outcome of oxaliplatin-based systemic treatment in advanced colorectal cancer patients. *Eur J Cancer* 2011;47 (3):369-74.
 29. Loprinzi CL, Qin R, Dakhil SR, et al. Phase III randomized, placebo-controlled, double blind study of intravenous calcium and magnesium to prevent oxaliplatin induced sensory neurotoxicity (N08CB/Alliance). *J Clin Oncol* 2014;32(10):997-1005.
 30. Jordan B, Jahn F, Beckmann J, Unverzagt S, Muller-Tidow C, Jordan K. Calcium and magnesium infusion for the prevention of oxaliplatin-induced peripheral neurotoxicity: a systematic review. *Oncology* 2016;90(6):299-306.
 31. Lynch ME, Cesar-Rittenberg P, Hohmann AG. A double-blind, placebo-controlled, crossover pilot trial with extension using an oral mucosal cannabinoid extract for treatment of chemotherapy-induced neuropathic pain. *J Pain Symptom Manage*. 2014;47(1):166-73.
 32. Hanai A, Ishiguro H, Sozu T, et al. Effects of Cryotherapy on Objective and Subjective Symptoms of Paclitaxel-Induced Neuropathy: Prospective Self-Controlled Trial, JNCI: Journal of the National Cancer Institute 2018;110(2):141-8.
 33. Beijers AJM, Bonhof CS, Mols F, et al. Multicenter randomized controlled trial to evaluate the efficacy and tolerability of frozen gloves for the prevention of chemotherapy-induced peripheral neuropathy. *Ann Oncol* 2020;31(1):131-6.
 34. Ruddy KJ, Le-Rademacher J, Lacouture ME, et al. Randomized controlled trial of cryotherapy to prevent paclitaxel-induced peripheral neuropathy; an ACCRU trial. *Breast* 2019;48:89-97.
 35. McCarthy AL, Shaban RZ, Gillespie K, Vick J. Cryotherapy for docetaxel-induced hand and nail toxicity: randomised control trial. *Support Care Cancer* 2014;22(5):1375-83.
 36. Griffiths C, Kwon N, Beaumont JL, Paice JA. Cold therapy to prevent paclitaxel-induced peripheral neuropathy. *Support Care Cancer* 2018;26(10):3461-9.
 37. Sundar R, Bandla A, Tan SS, Liao LD, et al. Limb Hypothermia for Preventing Paclitaxel-Induced Peripheral Neuropathy in Breast Cancer Patients: A Pilot Study. *Front Oncol* 2017;6:274.
 38. Bandla A, Tan S, Kumarakulasinghe NB, et al. Safety and tolerability of cryocompression as a method of enhanced limb hypothermia to reduce taxane-induced peripheral neuropathy. *Support Care Cancer* 2020;28(8):3691-9.
 39. Tsuyuki S, Senda N, Kanng Y, et al. Evaluation of the effect of compression therapy using surgical gloves on nanoparticle albumin-bound paclitaxel-induced peripheral neuropathy: a phase II multicenter study by the Kamigata Breast Cancer Study Group. *Breast Cancer Res Treat* 2016;160(1):61-7.
 40. Kanbayashi Y, Sakaguchi K, Ishikawa T, et al. Comparison of the efficacy of cryotherapy and compression therapy for preventing nanoparticle albumin-bound paclitaxel-induced peripheral neuropathy: A prospective self-controlled trial. *Breast* 2020;49:219-24.
 41. El-Fatraty BM, Ibrahim OM, Hussien FZ, Mostafa TM. Role of metformin in oxaliplatin-induced peripheral neuropathy in patients with stage III colorectal cancer: randomized, controlled study. *Int J Colorectal Dis* 2018;33(12):1675-83.

42. Mariani G, Garrone O, Granetto C, et al. Oxaliplatin induced neuropathy: could gabapentin be the answer? *Proc Am Soc Clin Oncol* 2000;19:609.
43. Rao RD, Michalak JC, Sloan JA, et al. Efficacy of gabapentin in the management of chemotherapy-induced peripheral neuropathy – A phase 3 randomized, double-blind, placebo-controlled, crossover trial (NOOC3). *Cancer* 2007;110(9):2110–8.
44. Saif MW, Syrigos K, Kaley K, Isufi I. Role of pregabalin in treatment of oxaliplatin induced sensory neuropathy. *Anticancer Res* 2010; 30(7):2927–33.
45. Durand JP, Deplanque G, Montheil V, et al. Efficacy of venlafaxine for the prevention and relief of oxaliplatin-induced acute neurotoxicity: results of EFOF, a randomized, double-blind, placebo-controlled phase III trial. *Ann Oncol* 2012;23(1):200–5.
46. Zimmerman C, Atherton PJ, Pachman D, et al. MC11C4: a pilot randomized, placebo-controlled, double-blind study of venlafaxine to prevent oxaliplatin-induced neuropathy. *Support Care Cancer* 2016;24(3):1071–8.
47. Farshchian N, Alavi A, Heydarheydari S, Moradian N. Comparative study of the effects of venlafaxine and duloxetine on chemotherapy-induced peripheral neuropathy. *Cancer Chemother Pharmacol* 2018;82(5):787–93.
48. Hirayama Y, Ishitani K, Sato Y, et al. Effect of duloxetine in Japanese patients with chemotherapy-induced peripheral neuropathy: a pilot randomized trial. *Int J Clin Oncol* 2015;20(5):866–71.
49. Salehifar E, Janbabaie G, Hendouei N, Alipour A, Tabrizi N, Avan R. Comparison of the Efficacy and Safety of Pregabalin and Duloxetine in Taxane-Induced Sensory Neuropathy: A Randomized Controlled Trial. *Clin Drug Investig* 2020;40(3):249–57.
50. Coriat R, Alexandre J, Nicco C, et al. Treatment of oxaliplatin-induced peripheral neuropathy by intravenous mangafodipir. *J Clin Investigation* 2014;124(1):262–72.
51. Penz M, Kornek GV, Raderer M, Ulrich-Pur H, Fiebigler W, Scheithauer W. Subcutaneous administration of amfostine: a promising therapeutic option in patients with oxaliplatin-related peripheral sensitive neuropathy. *Ann Oncol* 2001;12(3):421–2.
52. Esfahani A, Somi MH, Ayromlou H, et al. The effect of n-3 polyunsaturated fatty acids on incidence and severity of oxaliplatin induced peripheral neuropathy: a randomized controlled trial. *Biom Res* 2016;4:13.
53. Cassidy J, Bjarnason GA, Hickish T et al. Randomized double blind placebo controlled phase III study assessing the efficacy of xaliproden in reducing the cumulative peripheral sensory neuropathy induced by oxaliplatin and 5-FU/LV combination (FOLFOX4) in first line treatment of patients with metastatic colorectal cancer. *J Clin Oncol* 2006;24(Suppl):18S.
54. Liu MY, Huang XE. Effects of analgescine on oxaliplatin-induced neurotoxicity in patients with gastrointestinal cancer. *Asian Pacific J of Cancer Prevention* 2015; 16(10):4465–8.
55. Kono T, Hata T, Morita S, et al. Goshajinkigan oxaliplatin neurotoxicity evaluation (GONE): a phase 2, multicenter, randomized, double-blind, placebo-controlled trial of goshajinkigan to prevent oxaliplatin-induced neuropathy. *Cancer Chemother Pharmacol* 2013;72(6):1283–90.
56. Liu Y, Zhu G, Han L, Liu J, Ma T, Yu H. Clinical study on the prevention of oxaliplatin induced neurotoxicity with guilongtongluofang: results of a randomized, double-blind, placebo-controlled trial. *Evidence Based Complementary and Alternative Medicine* 2013;54:1217.
57. Molassiotis A, Suen LKP, Cheng HL, et al. A Randomized Assessor-Blinded Wait-List-Controlled Trial to Assess the Effectiveness of Acupuncture in the Management of Chemotherapy-Induced Peripheral Neuropathy. *Integrative Cancer Therapies* 2019.
58. Lu W, Giobbie-Hurder A, Freedman RA, et al. Acupuncture for Chemotherapy-Induced Peripheral Neuropathy in Breast Cancer Survivors: A Randomized Controlled Pilot Trial. *Oncologist* 2020;25(4):310–8.
59. De Gramont A, Cervantes A, Andre T. OPTIMOX study: FOLFOX7/LV5FU2 compared to FOLFOX4 in patients with advanced colorectal cancer. *J Clin Oncol* 2004;22:3525.
60. Grothey A, Hart LL, Rowland KM, et al. Intermittent oxaliplatin administration and time-to-treatment failure in metastatic colorectal cancer: Final results of the phase III CONCEPT trial. *J Clin Oncol (Meeting Abstracts)* 2008;26(15):4010.
61. Loprinzi CL, Lacchetti C, Bleeker J, et al. Prevention and Management of Chemotherapy-Induced Peripheral Neuropathy in Survivors of Adult Cancers: ASCO Guideline Update. *J Clin Oncol* 2020;38(28):3325–48.
62. Vas PR, Sharma S, Rayman G. Distal Sensorimotor Neuropathy: Improvements in Diagnosis. *Rev Diabet Stud* 2015;12(1-2):29–47.

RESEARCH ARTICLE

THE RESILIENCE OF ONCOLOGISTS DURING THE EARLY TIME OF THE COVID-19 PANDEMIC

A. Lasagna ¹, S. Secondino ¹, F. Agustoni ¹, T. Monaco ¹, I. Imarisio ¹, A. Pagani ¹, G. Rizzo ¹, R. J. Tancredi ¹, E. Pozzi ¹, E. Ferraris ¹, S. Chiellino ¹, C. Gandini ¹, A. Ferrari ¹, S. G. Brugnattelli ¹, P. Pedrazzoli ^{1,2}

¹ Medical Oncology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

² Department of Internal Medicine and Medical Therapy, University of Pavia, Pavia, Italy

CORRESPONDING AUTHOR:

Angioletta Lasagna
Medical Oncology Unit
Fondazione IRCCS Policlinico San Matteo
piazzale C. Golgi 19
27100 Pavia, Italy
E-mail: a.lasagna@smatteo.pv.it
ORCID: 0000-0002-9611-1164

Doi: 10.48286/aor.2021.07

ABSTRACT

With the emergence of COVID-19, Oncologists have had to face up to the challenge of continuing active treatments without compromising patients and healthcare personnel's safety.

We introduced a double-step triage strategy (by phone and on site) for cancer patients in order to identify patients at risk of COVID-19 and to avoid their admission to our Oncology Unit.

From February 24th to April 7th 2020, we performed 819 phone calls, leading to the authorization of 788 accesses (312 patients) to the outpatient clinic. 26 patients were kept at home, 23 managed with symptomatic treatments and 3 hospitalized for

suspected COVID-19. At the second triage level, 5 patients weren't admitted to the Outpatient clinic for respiratory distress.

None of the 58 healthcare workers were infected by SARS-CoV-2.

The surveillance strategy was carried on according to hospital indications which opted to do the screening only of people reporting symptoms because an active strategy was not feasible at that time.

Our practical approach allows the identification of patients at risk of COVID-19 infection and appears effective in maintaining cancer care with high levels of safety.

KEY WORDS

COVID-19; cancer; triage; resilience; management strategy.

IMPACT STATEMENT

A simple double-step triage strategy has been useful in maintaining cancer patient and health care worker safety during COVID-19 emergency in Italy.

INTRODUCTION

Italy's COVID-19 outbreak originated in Southwest Lombardy, on February 21st, 2020. The Fondazione IRCCS Policlinico San Matteo in Pavia, the nearest and largest teaching hospital near Codogno, was involved in the management of the outbreak from the start, undergoing a rapid and thorough reorganization (1, 2). In this emergency, the behavior of health care workers had to follow the risk management strategy of "resilience", a term used to define the ability to face a new situation by improving management skills (3). Also the oncologist community suggested to adapt the patient management in order to assist cancer patients in the safest way by introducing methods to improve a careful evaluation of every single patient to optimize oncological treatment (4). Practically, the oncologists needed to perform a substantial quality improvement focused to avoid nosocomial COVID 19 spread. According to these suggestions, and moving from a Deming cycle (Plan – Do – Check – Act), we planned (P) and put into practice a simple and safe triage protocol (D) aimed to screen each patient before his admission into Day Hospital or Ward spaces which had to remain COVID-19 free. This paper describes the obtained results (Check) to verify which improvements are needed (A).

MATERIALS AND METHODS

The outbreak of COVID-19 required an immediate reorganization of our workflow to minimize the risk of contamination. Before this emergency, there were no specific procedures to evaluate patients before admission for active treatment and there were no epidemiological checks.

From February 24th, we chose to start a protocol based on a double-step triage strategy for cancer patients, already under treatment or newly diagnosed, consisting of:

- first step: a phone call the day before active therapy or admission;
- second step: a clinical evaluation before the admission to the outpatient and inpatient wards on the day of the treatment.

The phone call was done by an experienced clinician the morning before the scheduled access in order to evaluate the clinical conditions of the patient and of the members of his/her family by asking information about the presence of signs/

symptoms as detailed in **table I**.

Every call lasted about 15 minutes and was preferably directed to the patient and not to the caregiver or to a member of the family to make sure that the symptoms were really reported and not missed.

This assessment took account of signs and symptoms potentially related to the underlying disease or treatment toxicity.

Moreover, the clinician asked each patient if, within the previous 72 hours, he/she had been into known outbreak areas (for the first period of epidemic), or had had direct contact with people known to have been affected by COVID-19 or with people currently in quarantine. The same questions were addressed also to the patient's relatives, to identify potentially infectious close contacts.

The questionnaire was modified according to relevant information on COVID-19 published in the medical literature (e.g., after the alert on anosmia and dysgeusia as consequence of COVID-19 (5)) and to the local protocol management (6).

In the presence of symptoms potentially related to COVID-19 infection, the patient was invited to stay at home, and a symptomatic treatment was suggested. Daily phone monitoring was implemented and, in cases of worsening of clinical status, the patient was signaled to the general practitioner for clinical evaluation at home and eventually referred to the regional Emergency Medical System (EMS) for evaluation for hospital admission (7), according to standard protocols of outpatient management.

The second triage level was performed before the patient entered either the day hospital or the inpatient ward by the nurse case manager and a physician, both wearing personal protection equipment (PPE) as suggested by WHO guidelines (8). This triage consisted of a new evaluation of clinical state by measuring body temperature and evaluating possible signs and symptoms of respiratory infections. This procedure aimed at a more careful examination of patients to reinforce what emerged at the first triage level. Both in the triage area and in the therapy rooms, a security distance of at least 120 cm was rigorously observed, and every patient was trained to wear a surgical mask and shoe covers, and to disinfect, at least at admission and before discharge, the hands with an hydro-alcoholic gel. Patients who were hospital admitted, in case of fever or other suspicious symptoms, underwent

SIGNS & SYMPTOMS PRESENT AT THE TIME OF THE CALL OR 2 WEEKS BEFORE	YES	NO
Temperature ≥ 37.3 °C	☐	☐
Gastrointestinal Symptoms (nausea/vomiting/diarrhea)	☐	☐
Respiratory symptoms (cough/nasal discharge, dyspnea)	☐	☐
Anosmia	☐	☐
Ageusia	☐	☐
Asthenia	☐	☐
Conjunctivitis	☐	☐
Headache	☐	☐
Myalgia/arthralgia	☐	☐
Patients' relatives with same symptoms	☐	☐
NUMBER OF PATIENTS REPORTING SIGNS/SYMPTOMS		
Patients with 1 sign/symptom n. 15		
Patients with 2-3 signs/symptoms n. 10		
Patients with > 3 signs/symptoms n. 1 (Fever, Cough, Anosmia)		
Symptomatic patients' relatives n. 1 (Fever)		

Table 1. First step triage questions asked by phone to patients the day before admission and number of patients reporting signs/symptoms.

a nasopharyngeal swab for SARS-CoV 2, an X-ray of the thorax and blood exams. In case the swab was negative, but X-ray was doubtful for a diagnosis of interstitial pneumonia, patients were not allowed to entry in the ward. With the aim of reducing social interaction, patient's relatives were not allowed to enter the hospital area.

Healthcare workers at the second level triage position and involved in the direct care of patients used the WHO-suggested PPE: eye protection (goggles), liquid-repelling gowns, double gloves, a class-2 filtering face-piece respirator (FFP2). Workers inside the Day Hospital room were equipped with surgical mask, goggles, not waterproof gowns and, obviously, gloves. The aim was to supply each worker with standard protective equipment for each work-shift.

Cleaning procedures have been also implemented and standardized; in particular, ward surfaces were cleaned every day with sodium hypochlorite in terminal sanitation (9, 10). Every day, a careful check of the procedure was made, with the aim of revealing any deviation from the protocol. Furthermore, the people wearing PPE followed refresher-training sessions on their use.

This brief report has been written following SQUIRE 2.0 framework as suggested by quality improvement guidelines.

RESULTS

From February 24th to April 7th 2020, we have performed 819 phone calls, leading to the authorization of 788 accesses (312 patients) to the outpatient clinic for active treatments. In the same period, one year ago, without any specific triage procedure, we recorded 820 Day hospital accesses. Twenty-six patients (8.3%) with fever (> 37.3 °C) and/or other symptoms were kept at home and managed by repeated telephone calls; 3 patients were subsequently hospitalized for suspected COVID-19, while 23 were managed at home with symptomatic treatments and antibiotics.

At the second triage level, 5 patients presented persistent fever or respiratory distress before being admitted to the Outpatient clinic, 2 of them were tested PCR-positive.

Overall, 5 triaged patients (3 at the first step and 2 at the second one) were hospitalized and proved

positive for SARS-CoV-2 by nasal swab. All patients experienced a course of hospital stay, treated with steroids, heparin and antibiotics, and were discharged alive.

In the same period 177 patients were admitted to the inpatient ward: 89 for cancer treatments, including 5 with germ cell tumors undergoing stem cell transplantation programs, 88 for diagnostic procedures or for supportive measures. As of April 7th, none was found to be COVID-19-positive and both outpatient and inpatient areas were still COVID-19 free. Moreover, no healthcare workers became infected by SARS-CoV-2.

The healthcare staff consisted of 20 doctors and 38 nurses and other people dedicated to patient assistance. The surveillance strategy was carried on according to hospital indications which opted to do the screening of people reporting symptoms because an active strategy was not feasible at that time.

The main characteristics of the patients stopped at the two triage levels are shown in the **table II**.

DISCUSSION

During the COVID-19 pandemic, oncologists were between Scylla and Charybdis (11): they had to protect both patients and themselves against the risk of infection and, at the same time, allow their patients to receive appropriate diagnostic work-ups and curative therapies (12).

So far many authors have reported the importance of organizing patient flow, including adopting the strategy of telemedicine, to minimize the contact between them (13-15). The approach described in the present single-institution experience, based on a simple double-step triage strategy, allows the identification of patients at risk for active COVID-19 infection, and avoids their admission to the outpatient clinic and inpatient ward. As compared with the pre-screening protocol, we arranged our activity without substantially reducing the normal "pre-COVID" level, and without the need of extra resources. During this emergency some health activities were suspended or drastically reduced, such as the follow-up visits which represent a significant commitment "in peacetime" in terms of time worked. Also first contact visits diminished as consequence of the reduction of surgical and diagnostic procedures. Some very frail patients were hospitalized to receive active oncological treatments or invasive diagnostic procedures and to avoid to expose them

TYPE OF TUMOR	NUM PATIENTS	%
Lung Cancer	11	36
Breast cancer	6	20
Colon cancer	6	20
Melanoma	2	6
Gastric cancer	1	3
Pancreatic cancer	1	3
Prostate cancer	1	3
Thymic carcinoma	1	3
Ovaric cancer	1	3
Renal cancer	1	3
TYPE OF ONCOLOGICAL TREATMENT	NUM PATIENTS	%
Chemotherapy	24	78
Immunotherapy	5	16
Targeted therapy	2	6
SETTING OF TREATMENT	NUM PATIENTS	%
Neoadjuvant	0	0
Adjuvant	5	16
Metastatic	26	84
COMORBIDITIES	NUM PATIENTS	%
Hypertension	16	52
Type 2 Diabetes	6	19
BPCO/Asthma	4	13
Coronary heart disease	4	13
Obesity	3	10
HCV/HBC	2	6
HIV	0	0
No comorbidities	10	32
≥ 1 comorbidity	21	68
SEX	NUM PATIENTS	%
Male	18	58
Female	13	42
AGE (YEARS)	NUM PATIENTS	%
18-29	0	0
30-39	1	3
40-49	5	16
50-59	9	29
60-69	8	26
70-79	7	23
≥ 80	1	3

Table II. Patients' characteristics not admitted in Day Hospital (first step and second step).

to COVID-19 infection during their stay in various areas of the hospital.

This study has main limitations, consisting in the lack of a control and of the possibility to compare it with the approach of other Hospitals in the same area: some Hospitals became COVID at all and

the Hospitals that remained COVID free adopted completely different protocols. Moreover, we did not put in place protocols like telemedicine; in fact they were reserved to the management of patients remaining at home and were not enough to screen patients needing a hospital access.

CONCLUSIONS

Our approach put successfully into practice the capacity to adapt our management to a global health emergency ("resilience") and to maintain high levels of safety for patients and health care workers as demonstrated by the absence of COVID-19 infections among patients and health care workers. According to the guidelines of improving quality in healthcare, this kind of strategy is fully sustainable even in a setting where the availability of swab and serologic tests is limited. Moreover, even where diagnostic methods are available, combining a clinical and virologic approach may be useful in case of oth-

er pandemic waves when the capacity of the health-care services may be once again overcrowded.

ACKNOWLEDGEMENTS

We are indebted with Professor Raffaele Bruno and the medical staff of Infectious Diseases Unit for the cooperation in organizing the management strategy.

FUNDINGS

This work was partially supported by Ricerca Corrente grant no 08067619, Fondazione IRCCS Policlinico San Matteo.

CONFLICTS OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

1. Asperges E, Novati S, Muzzi A, et al. Rapid response to COVID-19 outbreak in Northern Italy: how to convert a classic infectious disease ward into a COVID-19 response centre. *J Hosp Infect* 2020;105(3):477-9
2. Perlini S, Canevari F, Cortesi S, et al. Emergency Department and Out-of-Hospital Emergency System (112-AREU 118) integrated response to Coronavirus Disease 2019 in a Northern Italy centre. *Intern Emerg Med* 2020;15(5):825-33.
3. Mitchell T, Harris K. Resilience: A risk management approach. Available at: <https://www.odi.org/sites/odi.org.uk/files/odi-assets/publications-opinion-files/7552.pdf> Accessed: 07/04/2020.
4. Italian Association of Medical Oncology Statement. Available at: <https://www.aiom.it/comunicato-aiom-per-emergenza-corona-virus/>. Accessed: 07/04/2020.
5. Lechien JR, Chiesta-Estomba CM, De Siati DR, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol* 2020;277(8):2251-61.
6. Fondazione IRCCS Policlinico San Matteo. COVID Management Guidelines. Available at: <http://intranet.sanmatteo.org/site/home/sistema-qualita/protocolli-aziendali/mcs---modelli-comportamentali-standardizzati/docCatdocumenti-gestione-covid-19.1001181.1.50.1.all.html> Accessed: 07/04/2020.
7. Spina S, Marrazzo F, Migliari M, Stucchi R, Sforza A, Fumagalli R. The response of Milan's emergency medical system to the COVID-19 outbreak in Italy. *Lancet* 2020;395(10227):e49-e50.
8. WHO. Rational use of personal protective equipment (PPE) for coronavirus disease (COVID-19). Available at: https://apps.who.int/iris/bitstream/handle/10665/331498/WHO-2019-nCoV-IPCPPE_use-2020.2-eng.pdf Date: March 19, 2020. Accessed: 10/04/2020.
9. Colaneri M, Seminari E, Piralla A, et al. Lack of SARS-CoV-2 RNA environmental contamination in a tertiary referral hospital for infectious diseases in Northern Italy. *J Hosp Infect* 2020;105(3):474-6.
10. European Centre for Disease Prevention and Control (ECDC) Technical Report. Disinfection of environment in healthcare and non-healthcare settings potentially contaminated with SARS-COV-2. March 2020.
11. Lewis MA. Between Scylla and Charybdis - Oncologic Decision Making in the Time of Covid-19. *N Engl J Med* 2020;382(24):2285-7.
12. Tondo L. Life 'hanging by a thread' for Italian cancer patients in coronavirus crisis. *The Guardian* 7 Apr 2020.
13. Hanna TP, Evans GA, Booth CM. Cancer, COVID-19 and the precautionary principle: prioritizing treatment during a global pandemic. *Nat Rev Clin Oncol* 2020;17(5):268-70.
14. Lambertini M, Toss A, Passaro A, et al. Cancer care during the spread of coronavirus disease 2019 (COVID-19) in Italy: young oncologists' perspective. *ESMO Open* 2020;5(2):e000759.
15. Liu R, Sundaresan T, Reed ME, Trosman JR, Weldon CB, Kolevska T. (2020) Telehealth in Oncology During the COVID-19 Outbreak: Bringing the House Call Back Virtually. *JCO Oncol Pract* 2020;16(6):289-293.

RESEARCH ARTICLE

IMPACT OF DIFFERENT DOSING STRATEGIES OF NIVOLUMAB IN PATIENTS WITH SOLID TUMORS: ITALIAN SINGLE CENTER ANALYSIS

T. Gamba ^{1*}, A. Caglio ^{1*}, F. Sacchi ^{1*}, C. Paratore ^{1*}, G. Fazzina ^{2*}, V. Tagini ², M. Bellero ², S. Masucci ², F. Vignani ¹, G. Lacidogna ¹, C. Zichi ¹, G. Aimar ¹, D. Marino ¹, E. Sperti ¹, A. Gasco ², M. Di Maio ¹

¹ Department of Oncology, University of Turin, Ordine Mauriziano Hospital, Turin, Italy

² Hospital Pharmacy, Ordine Mauriziano Hospital, Turin, Italy

* Contributed equally

CORRESPONDING AUTHOR:

Massimo Di Maio
Department of Oncology
University of Turin
Ordine Mauriziano Hospital
largo Turati 62
10128 Turin, Italy
E-mail: massimo.dimaio@unito.it
ORCID: 0000-0001-8906-3785

Doi: 10.48286/aor.2021.08

ABSTRACT

Nivolumab is approved and reimbursed by Italian Drug Agency (AIFA) in several tumors, including non-small cell lung cancer, head & neck cancer and renal cell cancer. In May 2018, the original schedule (3 mg/kg every 2 weeks) - used in pivotal clinical trials demonstrating treatment efficacy - was replaced by a flat dose (240 mg every 2 weeks). Aim of this study was to identify the most cost-effective dosing strategy of nivolumab in a real-world setting. The primary endpoint of this analysis was the difference of nivolumab costs between the real scenario based on data from our hospital, and the hypothetical expenditure according to different simulated strategies of nivolumab dosing. The secondary endpoint was to report the economic savings associated with "drug day" and dose rounding strategies in the same scenario.

We collected data from patients treated with nivolumab at Mauriziano Umberto I Hospital in Turin, from January 2019 to August 2020. We ana-

lysed different dosing strategies (flat dose, weight-based-dose and hybrid), computed the cost of each one and compared them to the real expense. In addition, we performed sensitivity analysis modifying nivolumab price and mean patients' body weight. Among different dosing strategies, hybrid strategy was the most cost-effective approach, with a 11.7% saving compared to the real expense. Dose rounding and vial sharing minimized the drug waste. Applying our data to a hypothetical population of 1000 patients, savings associated with hybrid strategy could have covered 1423 additional treatment cycles. Hybrid strategy was confirmed the most-cost effective in all sensitivity analyses. Hybrid strategy was the most cost-effective strategy compared to the other dosing strategies (and compared to the real scenario, mainly based on fixed dose). Furthermore, this analysis demonstrated the importance of waste minimization policies, decreasing oncologic therapies costs.

KEY WORDS

Nivolumab; dosing strategies; flat-dose; hybrid strategy; cost-effective.

IMPACT STATEMENT

This analysis compared the economic impact of different dosing strategies of nivolumab, based on the results obtained in a single Italian center, and showing hybrid dose strategy as the most cost-effective one.

INTRODUCTION

Nivolumab is a fully humanized IgG4 antibody that enhances antitumor immune responses by blocking immune checkpoint and diminishing inhibitory signaling through the programmed death receptor-1 (PD-1) pathway (1). Randomized phase III trials CheckMate 017, 057, 025 and 141, testing nivolumab at the dose of 3 mg per kilogram of body weight (mg/kg) every 2 weeks (Q2W), had shown a better overall survival (OS) versus docetaxel in squamous and non-squamous metastatic non-small cell lung cancer (NSCLC) (2, 3), versus everolimus in advanced renal cell carcinoma (RCC) (4), and versus standard single-agent at investigator's choice in platinum-refractory squamous cell carcinoma of the head and neck (SCCHN) (5). Based on these results, as well as the results of other pivotal trials, nivolumab has been approved in many countries for treatment of several types of cancers, including previously-treated patients with metastatic NSCLC, RCC and SCCHN and patients with advanced melanoma. In Italy, nivolumab is reimbursed by Italian Medicines Agency (Agenzia Italiana del Farmaco, AIFA) for the aforementioned indications (6).

The drug had received the first approval for advanced melanoma in December 2014 by Food and Drug Administration (FDA) at a dose of 3 mg/kg Q2W, based on the results of the CheckMate-037 study (7). The dose of 3 mg/kg was originally selected based on nivolumab anti-tumor activity and safety data from a large phase Ib open-label dose-escalation study, conducted in patients with different advanced or recurrent malignancies. The drug showed a wide therapeutic index from 0.1 to 10 mg/kg, without a maximum tolerated dose (MTD) (8, 9). Clinical and pharmacodynamic data identified 3 mg/kg of nivolumab as the optimal regimen to guarantee the maximum efficacy and tolerability in different malignancies and to be tested in the following clinical trials. The use of a body-weight based schedule was justified by the intention of reducing variability in both drug distribution and elimination between patients.

In September 2016, US Food and Drug Administration (FDA) has modified the dosage regimen to the flat-dose of 240 mg Q2W (which is the equivalent dose for patients weighing 80 kg), followed by European Medicines Agency (EMA) in 2018. The transition to a fixed dose of nivolumab took place to allow a shorter drug preparation time, an easier administration and easier medical prescription of treatment regimens, and led to a waiting time reduction for patients (10). Following EMA decision, in May 2018 adoption of fixed dose was recommended by Italian Drug Agency. Consequently, all patients who started treatment after May 2018 received fixed dose, while patients already on treatment continued with the previous dosing strategy (3 mg/kg).

Several PD1/PD-L1 inhibitors, such as nivolumab, pembrolizumab and avelumab, were initially approved by regulatory agencies with a body weight-based dose, according to the standard practice of correlating drug clearance to body surface area. Nevertheless, several dose-findings studies have shown similar efficacy and safety between different dosing regimens through the assessment of pharmacokinetics data, exposure-response relationships and tolerability results (11, 12). Flat dose and body weight-based dose provide similar distribution and drug exposure, within a similar range of pharmacokinetic variability, ensuring additional support for switching dosing regimen (13). With regard to nivolumab, previous dose-response and exposure-response analyses showed overlapping safety and efficacy results, from either 3 mg/kg Q2W or 240 mg Q2W flat dose, which allows the use of flat dose as an effective therapeutic dosage for > 80 kg patients (8, 14-16). On the other side, pharmacodynamic trials has identified body weight-based dosing as the optimal regimen to provide the maximum efficacy and tolerability for several malignant disease (8, 9, 17) and subsequently in nivolumab pivotal trials (2-5), justify this schedule for < 80 kg patients.

However, several European and American studies have highlighted an increase in health expenditure with the adoption of flat dose. This discrepancy could be mainly attributed to the average weight of cancer patients in clinical practice, which is generally less than 80 kg (18-21). Several strategies have been proposed to reduce costs. Differently from flat dose, dose per kg of body weight, with different dose for each patient, is associated with a potential waste of drug. Waste minimization systems, such as the so-called “drug day”, in which the drug is administered only on certain days of the week, allow the reuse of drug residues in vials. Furthermore, the dose rounding allows to round off the theoretical calculated dose using the available vial sizes and avoiding to open another vial for a minimal amount of drug. Among the strategies proposed to reduce costs, the hybrid dosing strategy (using 3 mg/kg in patients below 80 kg and flat dose with 80 kg and above) allows to use the more convenient dosing in each patient (22).

This analysis was conducted to compare the economic impact of different nivolumab dosing strategies: the flat dose adopted in May 2018 as the “official” dosing strategy (240 mg Q2W), versus the body weight-based dose, used for previous patients and adopted in the pivotal trials (3 mg/Kg Q2W), versus the “hybrid” dosing strategy (consisting in the adoption of the 3 mg/Kg Q2W in patients with body weight < 80 kg and of the fixed dose 240 mg Q2W in patients with body weight ≥ 80 kg).

The aim of this study was to identify the most cost-effective dosing strategy and quantifying the potential economic savings in a real-world setting.

MATERIALS AND METHODS

In this single-center retrospective analysis, we collected expense and clinical data from patients with 3 different tumor types (metastatic NSCLC, advanced RCC, platinum-refractory SCCHN) treated with nivolumab at Mauriziano Umberto I Hospital in Turin, from January 2019 to August 2020.

The primary endpoint of this analysis was to describe the difference, in terms of nivolumab consumption and nivolumab costs, between the real expense data based on data of our hospital, and the hypothetical expenditure according to different simulated strategies of nivolumab dosing, in order to identify the most cost-effective approach. The secondary endpoint was to report the eco-

nomic savings allowed by “drug day” and dose rounding strategies in the same scenario.

The analysis is based on the real expense data of nivolumab at Mauriziano Umberto I Hospital. In addition, we estimated the hypothetical consumption and expenditure of nivolumab in a population of 1000 patients, using three different dosing strategies:

- body weight-based dose: 3 mg per kilogram of patient body weight, every 2 weeks;
- flat dose strategy: 240 mg for every administration, regardless of weight, every 2 weeks;
- hybrid strategy: body weight-based dose for patients with body weight below 80 kg, and flat dose for patients with body weight 80 kg and above.

For the simulated scenarios, we assumed that the distribution of patients’ characteristics (in particular, in terms of body weight) in the hypothetical population of 1000 patients is the same we observed in the series of patients treated at Mauriziano Umberto I Hospital. In addition, we assumed for our model the same proportion of patients still receiving the mg/kg dose in the period considered for the analysis, having started treatment before the regulatory change in the drug schedule. Finally, we assumed that the impact of pharmacological characteristics (available size vials, “drug day”, amount of dose rounding and drug wastes) is the same in our Institution and in the whole simulated population.

Pharmaceutical characteristics

Three different sizes of nivolumab vials were available: 4 ml, 10 ml and 24 ml. Each ml of solution contains 10 mg of drug, and each vial contains an overfill. Consequently, vials of 4 mL, 10 mL and 24 mL contain 45 mg, 110 mg and 247 mg of nivolumab respectively.

While in the fixed dose strategy the total dose is coincident with the whole content of vials, in body weight-based dose and hybrid strategies, a variable amount of nivolumab wastage may occur, because more than one vial may be required to reach the total dose for patients and the amount of drug in vials may not exactly match the required dose. In clinical practice, it is allowed to round up dosages (dose rounding strategy) to reduce the drug waste, assuming that the minimal reduction in the dose does not impact treatment efficacy. Our hospital Pharmacy allows a rounding of nivolumab dosage within the 5% of the theoretical dose.

While flat dose strategy does not produce drug waste, drug residuals and correlated costs were calculated for weight-based dose and hybrid strategies. Drug day system is used in our center to handle and minimize wastes.

SENSITIVITY ANALYSIS

Univariate sensitivity analysis was performed to control uncertainty of economic evaluation and to allow the generalizability in a larger population. After base-cost analysis of nivolumab, we re-calculated the results of different dosing strategies holding all parameters but modifying 2 variables, in 4 alternative scenarios. In this economic model, two main variables were identified: the price of nivolumab and the body weight of patient. The nivolumab price has been reduced by 30% from January 2020 in Italy. Until December 2019, the price of 1 mg of nivolumab was € 11,1 and the cost of one flat dose was € 2.664. From January 2020, the price of 1 mg has been reduced to € 7,69, with a total amount of € 1.845,6 for flat dose. Thus, we hypothesized two future scenarios with further 30% decreasing price compared to the current price: reduction of the current price from € 7,69 to € 5,39 (scenario I) and further reduction of 30% from € 5,39 to € 3,77 (scenario II). Regarding patient's body weight, in the study population we observed a large variety of weight-based dosages, from a minimal value of 130 mg to a maximum dose of 255 mg. The average weight-based dose was 192,9 mg corresponding to a price of € 2.026,3 in 2019 and € 1.403,8 in 2020. We then assumed two additional scenarios: scenario III considering a 5% increase in mean body weight and scenario IV considering a 5% decrease in mean body weight.

RESULTS

Baseline patient characteristics

33 patients were included in the study: 6 with RCC, 8 patients with SCCHN and 19 with NSCLC (**table I**). Nearly all patients had advanced disease: 24 patients had metastatic disease and 9 a locally advanced disease not amenable for curative treatment.

The mean weight of patients included in the analysis was 69,5 kg. Only 6 patients (18%) had a body weight greater than or equal to 80 kg, and most of the patients (53%) had a body weight between 60 and 80 kg.

	PATIENTS CHARACTERISTICS	N PTS (%)
MEAN AGE		68,3 y
SEX	Male Female	24 (73) 9 (27)
PERFORMANCE STATUS	ECOG 0 ECOG 1 ECOG 2	8 (24) 20 (60) 4 (12)
HISTOLOGY	NSCLC RCC SCCHN	19 (58) 6 (18) 8 (24)
STAGE	Locally advanced Metastatic disease	9 (27) 24 (73)
WEIGHT	Average weight ≥ 80 kg < 80 - > 60 kg ≤ 60 kg	69,5 kg 6 (18) 17 (53) 10 (30)
NIVOLUMAB	As 2 nd line As 3 rd line	25 (76) 7 (21)

Table I. Baseline patients' characteristics.

In the study population, treatment with nivolumab was always administered as a subsequent line after the failure of previous therapy: 25 patients (76%) as second line, 7 (21%) as third line and one (3%) as fourth line.

Regarding the nivolumab dose, about 70% of patients received flat dose and 30% received body weight-based dose. In fact, patients who had begun nivolumab before the regulatory recommendation of flat dose continued to receive nivolumab 3 mg/kg. Furthermore, from April 2020, new patients received the body weight dose due the approval of hybrid strategy by the Internal Pharmaceutical Commission of our Hospital.

We analysed the different alternative therapeutic strategies, computed the cost of each one and applied them to a hypothetical population of 1000 patients receiving nivolumab.

In the cost analysis, we estimated the potential expenditure for nivolumab for solid tumor treatment in the simulated population of 1000 patients using three different strategies (**table II**). This cost analysis included waste minimization system: drug day vial sharing and dose rounding. For flat dose strategy, the potential expenditure would be € 22.788.890,42, increasing the total spending by 4,6% compared to the real scenario (+ € 1.002.188,30). For body weight-based dose, the potential expenditure would be € 19.825.898,93,

	Δ % (VS REAL SCENARIO)	Δ N (VS REAL SCENARIO)	NIVOLUMAB EXPENSE DATA IN 1000 PATIENTS
REAL SCENARIO	-	-	€ 21.786.702,12
FLAT DOSE STRATEGY	+ 4,6	+ € 1.002.188,30	€ 22.788.890,42
BODY WEIGHT-BASED STRATEGY	- 9	- € 1.960.803,19	€ 19.825.898,93
HYBRID STRATEGY	- 11,7	- € 2.549.044,15	€ 19.237.657,97

Table II. Nivolumab expense data.

	Δ % (VS REAL SCENARIO)	Δ N (VS REAL SCENARIO)	NIVOLUMAB EXPENSE DATA + WASTE COST IN 1000 PATIENTS
REAL SCENARIO	-	-	€ 21.908.343,94
FLAT DOSE STRATEGY	+ 4 %	+ € 876.333,76	€ 22.784.677,70
BODY WEIGHT-BASED STRATEGY	- 8,3 %	- € 1.818.392,55	€ 20.089.951,39
HYBRID STRATEGY	- 11 %	- € 2.409.917,83	€ 19.498.426,11

Table III. Nivolumab expense data (without waste optimization).

with a saving of 9% (- € 1.960.803,19) compared to the real scenario. For hybrid strategy, the potential expenditure would be € 19.237.657,97, with a saving of 11.7% (- € 2.549.044,15).

Consequently, hybrid strategy could reduce the amount of nivolumab consumed causing cost savings by about 3% compared to the weight-based strategy. According to the cost analysis, hybrid strategy was the most cost-effective in every comparison. If we hypothesize that, differently from our Hospital, all 1000 patients actually received the flat dose, the adoption of body-weight based strategy would allow a 13% saving (€ 2.962.991,49), and the adoption of hybrid strategy would allow a 15.6% saving (€ 3.551.232,45).

We quantified the nivolumab waste and the corresponding cost during the observation period at Mauriziano Umberto I Hospital of Turin. Projecting these results into scenario population of 1000 patients, the cost of waste corresponded to € 121.641,82. The drug waste in the hybrid and body weight-based strategies would be larger than the real scenario by 105%, corresponding to an absolute increase of € 249.365,72. Flat dose would not create any drug waste, because of the administration of an entire vial per patient.

Transposing this result into a population of 1000 patients, the expenditure (excluding waste minimization policies, in order to estimate the waste impact) would have been € 21.908.343,94 (**table III**). There was no difference in drug waste between the hybrid and the body weight-based strategies. Indeed, the consumption and waste of nivolumab were the same in both strategies in patients with body weight less than 80 kg. In patients ≥ 80 kg, dosages of nivolumab were different, but the waste was similar due the using of dose rounding in body weight-based strategy.

In the economic model of nivolumab expense data in a population of 1000 patients, hybrid strategy was the most cost-effective approach regardless wastage cost, saving € 2.409.917,83.

Although hybrid and body weight-based strategies were the most economic, they produced the largest amount of drug waste. However, the use of dose rounding and vial sharing minimized the drug left unutilized, thus drug waste could be considered close to zero. Therefore, hybrid strategy with cost minimization systems could save € 2.549.044,15 in 1000 patients, confirming the aforementioned result (**table II**). Considering that the current price of nivolumab in Italy is € 7.69/mg, the total amount saved with the

HYBRID STRATEGY SAVING IN 1000 PATIENTS	CURRENT PRICE OF NIVOLUMAB	ADDITIONAL DRUG	ADDITIONAL CYCLES
€ 2.549.044,15	€ 7.69/mg	331.475,18 mg	1423 cycles

Table IV. Additional amount and cycles of Nivolumab using the savings obtained with hybrid strategy.

	€ 5,39/MG			€ 3,77 /MG		
	Δ % (COMPARED TO REAL SCENARIO)	Δ N (COMPARED TO REAL SCENARIO)	NIVOLUMAB EXPENSE DATA (SCENARIO I) IN 1000 PATIENTS	Δ % (COMPARED TO REAL SCENARIO)	Δ N (COMPARED TO REAL SCENARIO)	NIVOLUMAB EXPENSE DATA (SCENARIO II) IN 1000 PATIENTS
REAL SCENARIO	-	-	€ 15.270.523,33	-	-	€ 10.680.866,97
FLAT DOSE STRATEGY	+ 4,5 %	+ € 687.173,55	€ 15.957.696,88	+ 4,5 %	+ € 480.639,01	€ 11.161.505,98
BODY WEIGHT- BASED STRATEGY	- 9 %	- € 1.374.347,10	€ 13.896.176,23	- 9 %	- € 961.278,03	€ 9.719.588,94
HYBRID STRATEGY	- 11,6 %	- € 1.771.380,71	€ 13.499.142,63	- 11,6 %	- € 1.238.980,57	€ 9.441.886,40

Table V. Nivolumab expense data, with 2 different hypotheses of reduction in the drug price.

hybrid strategy could allow purchasing 331.475,18 mg of additional drug. Considering the median administration dose reported in our analysis at Umberto I Hospital, this amount of nivolumab could cover 1423 additional cycles (**table IV**).

A further reduction in the price of nivolumab would not change the results of our study. Assuming the hypothesis of a 30% price reduction of nivolumab from € 7,69 to € 5,39 per mg (scenario I, **table V**), the results would not differ from the baseline analysis. Considering an expense data of € 15.270.523,33 for real scenario, hybrid strategy could save € 1.771.380,71, which was about 3% less than the expense with the body weight-based dose. In the scenario II (€ 3,77 per mg of nivolumab), flat dose strategy was the most expensive one and the other two strategies were more advantageous with few differences (**table V**). Thus, we can assume that the results observed in our analysis are valid and stable even if the price of nivolumab changes. Hybrid strategy was the most-cost effective in every alternative scenario of sensitivity analysis, giving robust and reproducible finding.

The second sensitivity analysis evaluated the variability of patients' body weight. We hypothesized

a reduction of 5% of patient's weight in scenario III and an increase of 5% in scenario IV (**table VI**). In both scenarios III and IV, hybrid strategy was the most convenient. For instance, it reduced the spending of 8,9% in scenario III, which corresponded to a saving of € 1.957.225,94 in 1000 patients. In flat-dose strategy, the total cost did not change because the amount of drug administered did not depend on body weight. These data were consistent with the results of our analysis, showing also an increase in savings with lower patients' body weight.

Therefore, the results of our study are robust even when the nivolumab price and patient's body weight change, and the hybrid strategy is confirmed the most cost-effective.

DISCUSSION

According to our analysis, the highest savings using different nivolumab schedules can be achieved with the hybrid strategy, and secondly with the body weight-based strategy. These approaches could allow an absolute reduction of required

	+ 5% OF BODY WEIGHT			- 5% OF BODY WEIGHT		
	Δ % (COMPARED TO REAL SCENARIO)	Δ N (COMPARED TO REAL SCENARIO)	NIVOLUMAB EXPENSE DATA (SCENARIO III) IN 1000 PATIENTS	Δ % (COMPARED TO REAL SCENARIO)	Δ N (COMPARED TO REAL SCENARIO)	NIVOLUMAB EXPENSE DATA (SCENARIO IV) IN 1000 PATIENTS
REAL SCENARIO	-	-	€ 21.991.302,73	-	-	€ 21.641.058,18
FLAT DOSE STRATEGY	+ 3,7 %	+ € 813.678,20	€ 22.804.980,93	+ 5,4 %	+ € 1.168.617,14	€ 22.809.675,32
BODY WEIGHT- BASED STRATEGY	- 5 %	- € 1.099.565,14	€ 20.891.737,59	- 12,8 %	- € 2.770.055,45	€ 18.871.002,73
HYBRID STRATEGY	- 8,9 %	- € 1.957.225,94	€ 20.034.076,78	- 14,8%	- € 3.202.876,61	€ 18.438.181,57

Table VI. *Nivolumab expense data, with 2 different hypotheses of mean patients' body weight.*

amount of drug (mg), and consequently an absolute reduction of costs compared to the real scenario. Our results showed a potentially remarkable cost saving in our centre during the period examined. Nevertheless, our simulations showed an absolute saving of € 2.549.044,15 per 1000 patients (11,7% less than the cost of nivolumab in the real scenario) adopting a hypothetical hybrid strategy and an absolute saving of € 1.960.803,19 (9,0% less than the real scenario) by the hypothetical use of a body weight-based dose strategy during the observation period. Whereby, the flat-dose strategy led to an increased cost. These findings are consistent with the results of the few studies available nowadays in literature about the impact of drug dosing on the costs in the immunotherapy setting (20-23). In particular, a pharmacoeconomic analysis conducted in the US in 2017 pointed out that the body weight-based strategy ensured a significant cost saving compared to flat-dose strategy, with similar safety and efficacy (24). In the European setting, a retrospective observation on small Italian population in 2019 came to the same conclusions (19). The results achieved are also supported by the inclusion of drug wastes into the calculations. We observed that even considering the costs of drug waste per each administration, the hybrid strategy remained the most cost-effective approach, with an absolute saving of € 2.409.917,83 per 1000 patients (11,0% less than the real scenario). Furthermore, our study showed that minimizing costs with drug day, vial sharing and dose rounding strategies allows a reduction of the drug waste and

a further increase of the economic saving ensured by the hybrid strategy. These strategies minimized the drug left unutilized, thus drug waste could be considered close to zero. Our results corroborate the findings of similar experiences of other centres (25-27). In particular, an Italian multicentre study published in 2018 highlighted that the costs of immunotherapy treatments were inferior in the hospitals where a drug day strategy was been routinely applied (27).

Therefore, if we regard that hybrid strategy with cost minimization systems could save € 2.549.044,15 per 1000 patients, considering the current price of nivolumab in Italy, we could purchase about 331.475,18 mg of additional drug. These data are very meaningful, because, assuming this magnitude of costs saving and the mean dose administered in our center, we could have administered 1423 further cycles of nivolumab during the observed period.

Another strong point of the present analysis is the consistence of results, confirmed by the sensitivity analyses. The hybrid strategy was the most advantageous approach regardless of patient's body weight and decreasing in nivolumab price. This last issue could be a very probable eventuality for the coming years, due the many therapeutic indications of nivolumab. These results were consistent in all the hypothesized scenarios, considering either gross costs and waste-adjusted costs, and even modifying all the variables included in sensitivity analyses. Conversely, flat-dose was reconfirmed the most disadvantageous even vary-

ing those parameters. Therefore, the results are robust and the hybrid scenario is confirmed the most cost-effective.

Definitely, the decreasing cost, waste-minimizing and sensitivity analyses mentioned above strengthen the validity and robustness of the economic evaluations presented.

Nevertheless, the analysis has some weaknesses. The main limitation of the present study lies in the exiguity of the sample size. We selected a small sample, that not necessarily is representative of larger populations. Instead, only three cancer types were considered in the analyses of Maurizio Umberto I Hospital. This restriction leads to several consequences.

First of all, the small numerosity prevent us from drawing conclusions concerning safety and efficacy outcomes of the different schedules and strategies mentioned, and concerning a comparison between them. Nevertheless, the plenty of evidences regarding this topic available in the literature partially obviate this issue: several trials have shown the comparable efficacy and safety profile between the flat-dose and the body weight-based dose of nivolumab in advanced RCC, NSCLC and HNSCC (14, 15, 28). So, every consideration in our analysis rely on this assumption. Moreover, it is critical to highlight that the present study was not designed with the purpose of investigate efficacy and safety outcomes.

Furthermore, because of the small sample size it is hard to evaluate appropriately a significant economic impact of these strategies in a large-scale. However, a study conducted in the US in 2018 showed the efficacy of dose rounding strategy even in medical centres caring less than 100 patients per year (29). Therefore, despite these limitations, the economic savings observed could be considered significant.

Then, our evaluation did not include variations in the real cost of nivolumab for single Italian regions and single hospitals, beyond the official price, and did not include the whole spectrum of direct charges for the National health service (including the costs of clinical visit and follow-up, and the time for drug preparation and administration) and of indirect charges burdening on the patients (including the travel costs and the economic implications of lost working days). A more complete and exhaustive analysis should include the evaluation of all these factors, but nonetheless this issue does not affect the validity of the results, because the

number of visits is the same for the different doses considered in the models, and the extra costs mentioned above can be considered relatively negligible when compared to nivolumab price.

Definitely, the present study shows impressive results, even if the analysis was restricted to few cancer types. These findings must impel us to explore these strategies in the widest possible setting. It is important to emphasize that even if the validity of these results could be potentially extended to other European healthcare systems, further analyses are required regarding to different contexts. Actually, there is a remarkably huge variability between different healthcare systems in terms of economic factors: different treatment setting of day hospital or hospitalization regimen, different organization of the healthcare facilities, availability of different size of vials, different number of patients treated (30). By the way, there is a clear advantage in exploring the above-mentioned strategies in all the European healthcare systems.

CONCLUSIONS

The present study highlights the relevant economic savings potentially associated with the introduction of hybrid strategy in the setting of nivolumab therapy compared to the current standard flat-dose schedule. Furthermore, the study shows the remarkable economic impact in terms of oncologic therapies cost reduction through the use of waste minimization policies, such as dose rounding and drug day strategies. The present analysis provides also a possible perspective of economic implications and impact of a similar strategy on a larger scale.

In a landscape of rising costs of health care and of new anti-cancer drug discovery, especially concerning to future implementation of nivolumab and other immune-checkpoint inhibitors, it will be essential to identify and adopt strategies of costs minimization. In accordance with the results of the present analysis, the whole system would benefit if the hybrid strategy replaces the flat dose for all cancer patients in treatment with nivolumab. Moreover, vial sharing and dose rounding should be implemented whenever possible.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12(4):252-64.
- Brahmer J, Reckamp KL, Waterhouse D, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015;373(2):123-35.
- Borghaei H, Paz-Ares L, Barlesi F, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015;373(17):1627-39.
- Motzer RJ, Escudier B, Castellano D, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015;373(19):1803-13.
- Ferris RL, Blumenschein JG, Worden F, et al. Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med* 2016;375(19):1856-67.
- Gazzetta Ufficiale Serie Generale n. 224 del 26-09-2018. Regime di rimborsabilità e prezzo del medicinale per uso umano «Opdivo». Determina n. 1403/2018. (18A06153).
- Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* 2015;16(4):375-84.
- Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366(26):2443-54.
- Agrawal S, Feng Y, Roy A, Kollia G, Lestini B. Nivolumab dose selection: challenges, opportunities, and lessons learned for cancer immunotherapy. *J Immunother Cancer* 2016;4(1):72.
- Zhao, X, Ivaturi, V, Gopalakrishnan, M. Abstract CT101: a model-based exposure-response (ER) assessment of a nivolumab (NIVO) 4-weekly (Q4W) dosing schedule across multiple tumor types. *Cancer Res* 2017;77 (Suppl 13). Doi: 10.1158/1538-7445.AM2017-CT101.
- Chatterjee M, Turner DC, Flotten O, et al. Systematic evaluation of pembrolizumab dosing in patients with advanced non-small cell lung cancer. *Ann Oncol* 2016;27(7):1291-8.
- Novakovic AM, Wilkins JJ, Dai H, et al. Changing Body Weight-Based Dosing to a Flat Dose for Avelumab in Metastatic Merkel Cell and Advanced Urothelial Carcinoma. *Clin Pharmacol Ther* 2020;107(3):588-96.
- Freshwater T, Kondic A, Stone JA et al. Evaluation of dosing strategy for pembrolizumab for oncology indications. *J Immunother Cancer* 2017;5:43.
- A. Zhao X, Suryawanshi S, Hruska M, et al. Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3-mg/kg dosing regimen in patients with advanced tumors. *Ann Oncol* 2017;28(8):2002-8.
- Feng Y, Wang X, Roy A, et al. Nivolumab exposure-response analyses of efficacy and safety in previously treated squamous or nonsquamous non-small cell lung cancer. *Clin Cancer Res* 2017;23(18):5394-405.
- Bajaj G, Wang X, Agrawal S, Gupta M, et al. Model-Based Population Pharmacokinetic Analysis of Nivolumab in Patients With Solid Tumors. *CPT Pharmacometrics Syst Pharmacol* 2017;6(1):58-66.
- Bellesoeur E, Ollier MA, Blanchet B, et al. Is there an Exposure-Response Relationship for Nivolumab in Real-World NSCLC Patients? *Cancer* 2019;11(11):1784.
- Goldstein D. Weight-based dosing vs fixed dosing of pembrolizumab: an economic analysis. *Clin Adv Hematol Oncol* 2018;16(8):549-51.
- Giuliani J, Albanese V, Ponturo G, Bonetti A. Economic sustainability of nivolumab at flat dose for second-line treatment of metastatic non-small cell lung cancer in real life. *J Oncol Pharm Pract* 2019;25(8):2059-60.
- Bayle A, Besse B, Annereau M, Bonastre J. Switch to anti-programmed cell death protein 1 (anti-PD-1) fixed-dose regimen: what is the economic impact?. *Eur J Cancer* 2019;113:28-31.
- Hall E, Zhang J, Kim EJ, Hwang G, Chu G, Bhatta S, Reddy S. Economics of alternative dosing strategies for pembrolizumab and nivolumab at a single academic cancer center. *Cancer Med* 2020;9(6):2106-12.
- De Lemos ML, Kung C, Waignein S. Efficacy of nivolumab four-weekly dosing schedule based on body weight. *J Oncol Pharm Pract* 2019;25(4):961-3.
- Goldstein DA, Ratain MJ, Saltz LB. Weight-Based dosing of pembrolizumab every 6 weeks in the time of COVID-19. *JAMA Oncol* 2020;6(11):1694-5.

24. Goldstein DA, Gordon N, Michal Davidescu M, et al. A phamacoeconomic analysis of personalized dosing vs fixed dosing of pembrolizumab in firstline PDL1-positive Non-Small Cell Lung Cancer. *J Natl Cancer Inst* 2017;109(11).
25. Hess LM, Cui ZL, Li XI, Oton AB, Shortenhaus S, Watson IA. Drug wastage and costs to the healthcare system in the care of patients with non-small cell lung cancer in the United States. *J Med Econ* 2018;21(8):755-61.
26. Fasola G, Aita LM. Drug waste minimisation and cost-containment in Medical Oncology: two-year results of a feasibility study. *BMC Health Serv Res* 2008;8:70.
27. Damuzzo V, Russi A, Chiumente M, Masini C, et al. Optimization of resources by drug management: a multicentred web-administered study on the use of ipilimumab in Italy. *J Oncol Pharm Pract* 2018;25(4):787-92.
28. Kato R, Ikarashi D, et al. Analyses of nivolumab exposure and clinical safety between 3-mg/kg dosing and 240-mg flat dosing in Asian patients with advanced Renal Cell Carcinoma in the real-world clinical setting. *Transl Oncol* 2020;13(6):100771.
29. Chillari KA, Southward J, Harrigan N. Assessment of the potential impact of dose rounding parenteral chemotherapy agents on cost savings and drug waste minimization. *J Oncol Pharm Pract* 2018;24(7):507-10.
30. Buxton MJ, Drummond MF, Van Hout BA, et al. Modelling in economic evaluation: an unavoidable fact of life. *Health Econ* 2017;(3):217-27.

REVIEW

SAFETY MATTERS: THE TROUBLED AND FINALLY SUCCESSFUL STORY OF DIHYDROPYRIMIDINE DEHYDROGENASE PHARMACOGENETIC TEST IN CANCER PATIENTS

S. Crucitta ¹, F. Sciandra ¹, A. Cerbioni ¹, F. Cucchiara ¹, R. Arici ¹, C. Rafaniello ², A. Capuano ², R. van Schaik ³, R. Danesi ¹, M. Del Re ¹

¹ Unit of Clinical Pharmacology and Pharmacogenetics, Department of Clinical and Experimental Medicine, University Hospital of Pisa, Pisa, Italy

² Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy

³ Department of Clinical Chemistry, Erasmus University Medical Center, Rotterdam, Netherlands

CORRESPONDING AUTHOR:

Romano Danesi

Unit of Clinical Pharmacology and Pharmacogenetics

Department of Clinical and Experimental Medicine

University Hospital of Pisa

55 via Roma

56126 Pisa, Italy

E-mail: romano.danesi@unipi.it

ORCID: 000-0002-4414-8934

Doi: 10.48286/aor.2021.09

ABSTRACT

Pharmacogenetics investigates the molecular basis of inter-individual differences in drug metabolism and response. Sequence variations in genes encoding for metabolic enzymes may influence drug's pharmacokinetics and/or pharmacodynamics, resulting in reduced efficacy and/or adverse drug reactions. The dihydropyrimidine dehydrogenase (DPD) deficiency is a clear example of how gene variants may affect fluoropyrimidines metabolism, and its evaluation has been recently recommended from regulatory agencies for its implementations in clinical routine. This review provides a summary of pharmacogenetic research on DPD and fluoropyrimidines metabolism, and its involvement in adverse drug reactions.

KEY WORDS

DPYD; 5-FU; capecitabine; chemotherapy; pharmacogenetic.

IMPACT STATEMENT

Dihydropyrimidine dehydrogenase (DPD) is the major responsible for fluoropyrimidines metabolism and its deficiency may lead to life-threatening toxicities. Although the frequency of DPD deleterious variants is low, their screening is clinically relevant to avoid severe toxicities or death in patients treated with fluoropyrimidine-based chemotherapy. While for the most studied variants the dose reduction to apply is already well known, future researches are necessary to understand the role of other DPD mutations, including the c.2194G > A variant, for which interest data have been recently published.

INTRODUCTION

Fluoropyrimidines, including 5-fluorouracil (5-FU) and its prodrug capecitabine (1), are the backbone of chemotherapy regimens for treatment of solid tumors, such as breast (2), colorectal (3), head and neck (4), gastrointestinal (5) and pancreatic cancers (6). They can be used either alone or in combination with irinotecan, platin derivatives, cyclophosphamide, epirubicin and monoclonal antibodies (e.g., cetuximab and bevacizumab) (7-10).

Owing to the widespread use of these drugs, severe fluoropyrimidine-associated toxicity have been reported, including gastrointestinal toxicity (stomatitis, nausea/vomiting, diarrhea), skin toxicity (pigmentary abnormalities, conjunctivitis, hand-foot syndrome [HFS], skin rashes and hair loss in severe cases), haematological toxicity (neutropenia, leucopenia, anemia), and cardiac toxicity (arrhythmias and cardiac ischemia) (11). Neurologic abnormalities (cerebellar ataxia and changes in cognitive function) have also been reported in less than 1% of the population (11, 12). These toxicities may be the responsible of delays in drug administration or even treatment discontinuation, compromising its therapeutic benefit. Of note, capecitabine seems to display a different profile of toxicity compared to 5-FU, being characterized by a better tolerability, but higher incidence of HFS (13). Its oral administration has some advantages, particularly in patient's quality of life, and for this reason its use is becoming more diffuse in USA and Europe (14). Despite this, a number of patients develop severe, life-threatening toxicity due to fluoropyrimidine-related adverse events. It is well known that dihydropyrimidine dehydrogenase (DPD) deficiency is at the basis of this toxicity (15), therefore, over the past years a growing number of research addressed the clinical effects of genetic variants in the DPD gene (DPYD), claiming their screening as pre-emptive strategy in patients candidate to fluoropyrimidines treatment. This review provides a summary of pharmacogenetic research on DPD and fluoropyrimidines metabolism and its involvement in adverse drug reactions, including relevant information about clinical implementation of DPD testing.

Fluoropyrimidines: metabolism and mechanism of action

Over 80% of 5-FU is transformed to inactive metabolites into the liver, and about 5% is the responsible for the therapeutic effect (16). The DPD en-

zyme catabolizes the first step of 5-FU metabolism, transforming the drug into the dihydrofluorouracil (5-FDHU) metabolite. The 5-FDHU is converted to fluoro- β -alanine (FBAL) by two additional enzymes, the dihydropyrimidinease (DPYS) and the beta-ureidopropionase (UPB1) (17), to be lastly excreted by the kidneys. The remaining 5-FU is converted directly or indirectly into fluorouridine monophosphate (FUMP). The phosphorylation of FUMP leads to the formation of FUTP or FdUDP, which is subsequently phosphorylated or dephosphorylated into the active metabolites FdUTP and FdUMP, respectively. FdUMP inhibits the thymidylate synthase (TS), leading to the inhibition of DNA replication (18) (**figure 1**).

Fluoropyrimidines, by acting as analogues of uracil, are able to interfere with the DNA synthesis at different levels: 1) the antifolate 5-fluorodeoxyuridine monophosphate (5-FdUMP) covalently binds and inhibits TS, leading to a reduction of thymidine synthesis and of the DNA molecule; 2) the 5-fluorodeoxyuridine triphosphate (5-FdUTP) metabolite can be directly incorporated into genomic DNA causing damage; 3) the 5-fluorouridine triphosphate (5-FUTP) can be directly incorporated into genomic RNA causing damage (19).

DPYD variants conferring high risk for fluoropyrimidines toxicity

There is a link between a DPD deficiency and the occurrence of severe or life-threatening toxicity due to fluoropyrimidines treatment. Patients with a significant DPD deficiency, receiving standard dose of fluoropyrimidine result in overexposure of 5-FU, with development of severe haematological and gastrointestinal toxicities (20) (**figure 2**). In these cases, the toxicity often occurs early after the first cycles of chemotherapy and it is characterized by grade 4 (WHO) symptoms and potentially death. High-grade of diarrhoea, mucositis and neutropenia are the most frequent reported side effects (21). DPD enzyme is encoded by the DPYD gene (22), which is located on the human chromosomal region 1p22, and is composed of 23 exons, spanning 950 Kbs (23). Over 90 among single nucleotide polymorphisms (SNPs) and mutations, including also deletion/insertion, have been described in the DPYD gene, with relevant functional consequences on enzymatic activity for some of them (20). The most clinically relevant DPYD variants reported with statistically significant association with severe toxicity include: c.1236G > A (E412E; rs56038477, haplo-

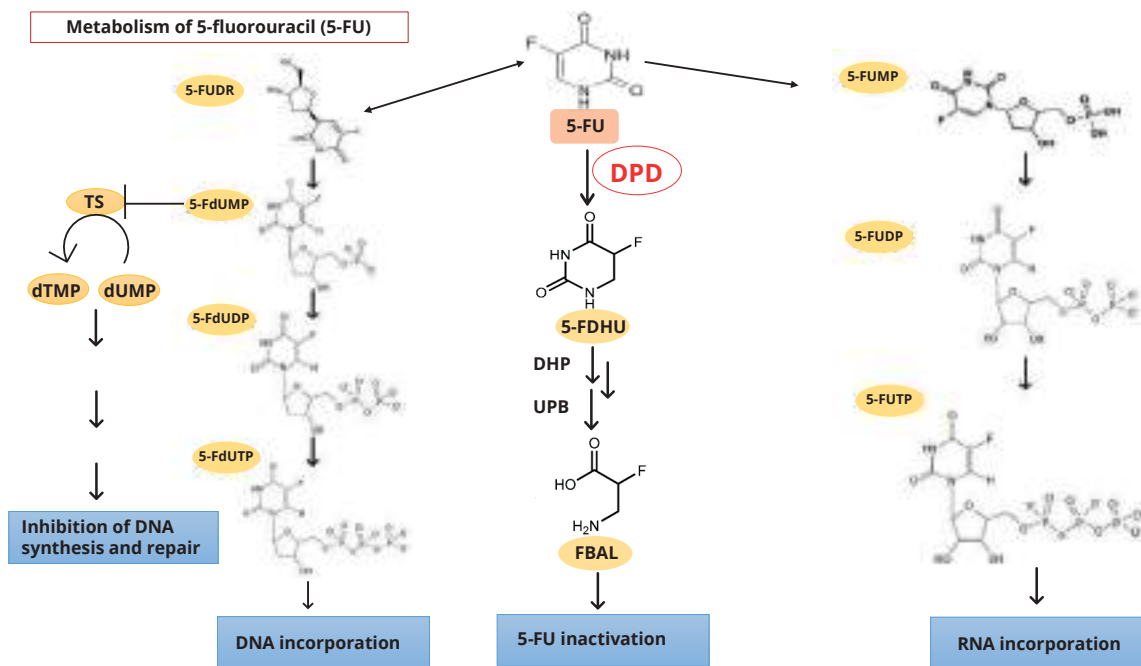


Figure 1. Metabolic pathway of 5-fluorouracil (5-FU).

DPD, dihydropyrimidine dehydrogenase; 5-FDHF, dihydrofluorouracil; DHP, dihydropyrimidine; UPB, beta-ureidopropionate; F-b-AL, fluoro-b-alanine, FdUDP, fluorodeoxyuridine diphosphate; FdUMP, fluorodeoxyuridine monophosphate; FdUTP, fluorodeoxyuridine triphosphate; FUTP, fluorouridine triphosphate; TS, thymidylate synthase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate.

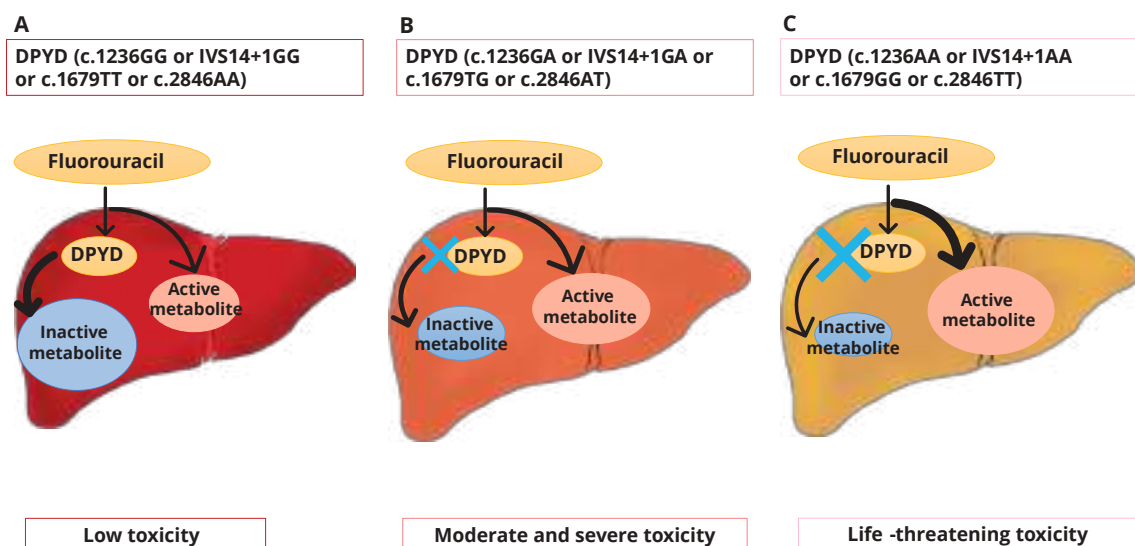


Figure 2. a. Schematic overview of fluoropyrimidine metabolisms and treatment related-toxicity in patients carrying wild-type; b. heterozygous or c. homozygous clinically relevant variants for DPYD gene.

type B3), DPYD*13 (rs55886062, c.1679T > G, I560S), DPYD*2A (rs3918290, c.1905 + 1G > A, IVS14 + 1G > A), c.2846A > T (rs67376798, D949V) (24-26). Of these variants, DPYD*13, DPYD*2A and c.2846A > T have the most deleterious impact on DPD activity, whereas c.1236G > A results in moderate reduction of DPD activity and consequent toxicity (26, 27).

The frequency of reduced DPD activity in the healthy population is estimated between 3-5% (28, 29). Indeed, a significant variability between ethnic subgroups was observed, and data obtained from phenotype and genotype analyses showed that Asian (30-32), African (33), and Caucasian (34) population present DPD deficiency at variable rates.

DPYD*2A is the most studied mutation of the DPYD gene. It is associated with a significantly reduced enzyme activity of 50% in heterozygous patients, and a complete deficiency of enzyme activity in homozygous subjects, causing a life-threatening toxicity due to 5-FU accumulation (35). This splice site mutation in intron 14 changes the invariant junction donor site from G to A. As a result, a deletion of the entire exon 14 occurs and the truncated protein is catalytically inactive (36). Allele frequencies of DPYD*2A have been described to differ between ~ 0.1 and 1.0% in African-American and Caucasian population, respectively (37-39). Similarly, DPYD *13 and c.2846A > T substitution have been reported to be associated with partial or total loss enzyme activity, in heterozygous or homozygous patients, respectively (24, 34, 40). DPYD*13 is characterised by an Ile560Ser amino acid change in the DPD binding domain, which lead to the destabilization of the protein (22, 41). In vitro study by Offer *et al.* showed that the homozygous expression of this variant resulted in a 75% reduction in enzyme activity respect to the wild-type (36). Allele frequencies of DPYD*13 were found to vary from 0.07 to 0.2% in the Caucasian population (38, 42). DPYD c.2846A > T results in an Asp949Val amino acid change localized near an iron-sulfur motif (41). Homozygous expression of the c.2846A > T variant results in 59% of activity compared to wild-type (37). Reported allele frequencies of c.2846A > T vary from 0.1 to 1.1% in African-Americans and Caucasians, respectively (34, 37, 38, 42). The synonymous variant c.1236G > A occurs in exon 11 and it is in complete linkage with haplotype B3 variants (c.483 + 18G > A, c.680 + 139G > A, c.959-51T > G and c.1129-5923C > G), resulting in a partial non-functional transcript, ranging from 44 to 50% of DPD activity for homozygous carriers (43, 44). The frequency of heterozygous patients in Caucasian population was reported to vary between 2.6 and 6.3% (44-47). Recently, a new variant, the DPYD*6 (rs1801160, c.2194G > A, p.V732I), has been associated with 5-FU related toxicity (27). Allele frequencies of DPYD*6 was found to vary from 1 to 7% in Caucasians, Asians and African Americans population (22, 48-50). However, conflicting results concerning the reduction of enzyme activity of this DPD variant have been published (36, 51, 52). Several studies published in literature highlighted the correlation between 5-FU toxicities and the above reported DPD variants (24, 38, 40, 53-57). A comprehensive pharmacogenetic analy-

sis on 5-FU toxicity was conducted by Rosmarin *et al.* in 927 colorectal cancer patients enrolled in the QUASAR2 trial (55). The authors tested candidate polymorphisms for their associations with capecitabine-dependent toxicity, including diarrhea, nausea/vomiting, stomatitis, neutropenia, thrombocytopenia, and HFS. DPYD c.2846T > A and DPYD*2A mutations were associated with capecitabine-related overall ($\geq$ grade 3) toxicity with a significant odds ratio of 5.51 ($p = 0.0013$) (55). Similarly, a meta-analysis by Terrazzino *et al.* highlighted a strong correlation between DPYD*2A and c.2846A > T variants and the development of high-grade toxicities (odds ratio 5.42, $p < 0.001$) (40), confirming the clinical validity of these SNPs as risk factors of 5-FU-related toxicities. A recent meta-analysis involving 7365 cancer patients with severe toxicity related to 5-FU treatment (fluorouracil, capecitabine, or tegafur-uracil as single agents, in combination with other anticancer drugs or radiotherapy) showed that DPYD*13 and c.1236G > A/HapB3 DPYD variants, in addition to DPYD*2A, and c.2846A > T, were independent predictors of severe gastrointestinal and haematological fluoropyrimidine-associated toxicity (24). Patients carrying the DPYD*2A, c.1236G > A/HapB3, DPYD*13 and c.2846A > T had a relative risk for toxicity of 2.9 (95%CI: 1.8-4.6), 1.6 (95%CI: 1.3-2.0), 4.4 (95%CI: 2.1-9.3) and 3.0 (95%CI: 2.2-4.1), respectively (24). In contrast, in a study conducted on 603 cancer patients treated with fluoropyrimidine-based chemotherapy as neo-adjuvant/adjuvant or as first-line setting, retrospectively tested for 8 DPYD polymorphisms (c.496A > G, *2A, *4, *5, *6, *13, c.1896 T > C, c.2846A > T), showed that the association between DPYD*13 variant and severe toxicity was not statistically significant, probably because of the low frequency of the mutation (0.3%) (58). However, the study found DPYD*2A and c.2846A > T significantly associated to grade ≥ 3 toxicity ($p = 0.003$, $p = 0.048$), including neutropenia, diarrhea, leukopenia, stomatitis and nausea/vomiting (58). The retrospective DPYD analysis of the pharmacogenetic study of the TOSCA trial, evaluated 10 DPYD variants for their associations with high-grade fluoropyrimidine-related adverse events in colorectal cancer patients, undergoing adjuvant fluoropyrimidine/oxaliplatin combination chemotherapy. The time-to-toxicity analysis highlighted the contribution of the DPYD*6 (rs1801160, c.2194G > A) variant to 5-FU toxicities, in particular, neutropenia (59). Similarly, in a secondary analy-

sis of the PETACC-8 trial, the DPYD*6 was significantly associated with high-grade adverse events (60). Recently, also evaluated the role of 8 DPYD variants (c.496A > G, c.1236G > A/HapB3, c.1601G > A, c.1627A > G, DPYD*13, c.1896T > C, DPYD*2A, DPYD*6, c.2846A > T) in a cohort of 1254 patients, treated with fluoropyrimidine-containing regimens. A significant association between DPYD*6 variant, in addition to DPYD*2A and c.2846A > T, with gastrointestinal and hematological toxicities was found. Moreover, the study compared the DPYD variants found in the cohort of 982 patients with toxicity, to a control group of 272 patients receiving standard doses of fluoropyrimidine-based therapies, who had no dose reduction, delay or discontinuation of therapy due to toxicity. The association between the most frequent DPYD polymorphisms c.496A > G, c.1601G > A, c.1627A > G, c.1896T > C and toxicity was not statistically significant, since those SNPs were also found in the control group (27). However, several studies highlighted the importance of other DPYD variants, including DPYD*9A, *3, and *4. DPYD*9A (rs1801265, c.85T > C) results in a Cys29Arg conversion in the crystal structure of the protein. Controversial results have been reported concerning the effect of this variant (61-64), as well as different effects in individuals of different ethnic background have been described. A study on Asian patients receiving fluorouracil-based chemotherapy, showed an association of the variant allele with treatment-related toxicity (65), however a reduced DPD activity was not observed when DPD activity was measured in blood samples (50). Indeed, studies in Caucasian patients did not find any effect of this variant on 5-FU clearance and toxicity (61, 66, 67). The DPYD*3 variant was reported at very low frequency (68), and has not been identified in any of the key studies of fluoropyrimidine toxicity or population studies (33, 48, 50, 69). The DPYD*4 (c.1601G > A, p.Ser534Asn) variant has been found at low frequencies of < 2% in Caucasians, Asians and African Americans (22, 48, 50). Controversial results have been published for this variant phenotype, with no correlation with DPD activity (48) or with case series where the variant was associated with severe fluorouracil-related toxicity (61, 70). Additional polymorphisms have been described in non-Caucasian population. Among African and American individuals, the common variant c.557A > G (rs115232898, p.Y186C) has been identified as a potential risk factor for 5-FU-related toxicity (71).

DPYD*5 (rs1801159, c.1627A > G, p.Ile543Val) variant, located in exon 13, is reported in the Asian population with the highest frequency rate (72). Researches identified DPYD*5 and DPYD*9A, together with DPYD*2A as the common frequent SNPs in the Chinese population (73, 74). Several other variants have been frequently associated with fluoropyrimidine toxicities, including, c.257C > T, c.496A > G (p.M166V), c.680G > A, c.1801G > C, c.1850C > T, c.1896T > C, and c.2509-2510insC (21, 75-78). Falvella *et al.* investigated the association between DPYD variants c.496A > G, c.1129-5923C > G, c.1896T > C and capecitabine-related toxicity in 64 metastatic colorectal cancer patients enrolled in phase II trials, showing a significant association between DPYD c.496A > G and high grade (> 3) adverse events, including diarrhoea and neutropenia (79). However, no association between c.1129-5923C > G and toxicity was found. Accordingly, the multivariate analysis reported by Lee *et al.*, failed to show a significant association of this DPYD variant with 5-FU related toxicity, suggesting a limited predictive value for severe toxicity to 5-FU-based chemotherapy (80). Similarly, Zhang *et al.* investigated the association between 4 DPYD SNPs (c.496A > G, c.1627A > G, c.2194G > A, c.*274T > C) and clinical outcomes of 362 Chinese gastric cancer patients treated with fluorouracil-based adjuvant chemotherapy. The authors found a significant association between c.1627A > G and clinical outcome after 5-FU-based regimens. However, no association between the occurrence of toxicities and the evaluated SNPs was found (81). Several studies identified novel rare DPYD variants associated with fluoropyrimidine toxicity (75, 77, 82-85). Three novel DPYD variants (c.2509-2510insC, c.1801G > C, and c.680G > A), together with other known sequence variants, were detected in patients experiencing unexpected life-threatening toxicities after treatment with 5-FU or capecitabine. The non-synonymous nature of these variants result in a conformational changes of the enzyme affecting DPD activity (76). Similarly, a treatment-related death case regarding a breast cancer patient treated with capecitabine and trastuzumab was reported (75). Four DPYD variants after sequencing analysis were identified: the above-mentioned c.496A > G and c.2194G > A, c.257C > T, causing deficient enzyme activity, and the c.1805C > T, leading to threonine-methionine amino acid change associated with reduced DPD activity (75). This report highlighted the dangerous effect of the combina-

tion of new DPYD variants as a possible cause of death in a patient treated with fluoropyrimidines. Likewise, van Kuilenburg *et al.* showed the effect of 2 DPYD variants (c.61C > T and *2A), located on different alleles, as responsible of lethal toxicity after the administration of 5-FU. The 2 DPYD mutations, presented in heterozygous manner, caused a complete deficiency of enzyme activity (86). Recently, Ly *et al.* reported a case of a patient with a rare variant of unknown significance in DPYD (rs755416212, c.704G.A, p.R235Q) who had a life-threatening toxicity capecitabine-related. An in silico tool (DPYD-Varifier (87)) confirmed the deleterious impact on the enzyme activity, and in vitro analysis confirmed the significantly reduced DPD activity by 88% compared to the wild-type DPD (88). Interestingly, the effect of the DPYD p.R235Q variant was similar to the p.R235W (37), previously detected in a patient with DPD deficiency (62), and CPIC's guidelines for DPYD genotype-guided dosing incorporated this variant as a non-functional allele (26). DPYD-Varifier was also used by Shrestha *et al.* to predict the deleterious impact of DPYD c.394A > G (p.T132A) variant, discovered in a rectal cancer patient who experienced severe FU-associated toxicity during neoadjuvant therapy with capecitabine. In vitro and ex vivo approaches have been used to validate the deleterious function of this new variant. Based on these results the authors determined an activity score for the patient that was used to calculate a safe adjusted dose of FU for adjuvant therapy (83). Nevertheless, no confirmatory studies have been reported and their association with toxicity remains unclear.

Different approaches for DPD screening

DPD deficiency can be tested out by using different techniques, including the phenotype test (direct or indirect measurement of enzymatic activity) or by genotype testing (searching for the main functional polymorphisms of the DPYD gene). The phenotypic approach evaluates the activity of the enzyme (89). Several DPD activity measurement have been tested, including dosage in peripheral mononuclear blood cells (28, 90), and dosage by physiological ratio dihydrouracil/uracil (UH2/U) in plasma, serum, saliva, or urine (66, 91-94). Nevertheless, the application of these methods in clinical practice is complex, due to the special technical equipment and expertise required (95, 96). Indeed, DPD activity in peripheral blood mononuclear cells (PBMCs) may be influenced by several factors, including sam-

pling and storage and cell heterogeneity (97).

Conversely, the genotyping approach is more reliable, fast and there are fewer factors that can influence the result. The advantages of a genotyping test include that only a small blood sample is required for DNA extraction, and particular precautions (such as storage condition) are not necessary. The detection of DPYD genetic variants may be carried out by multiple technologies, usually available in laboratories involved in molecular analysis, such as Sanger Sequencing, Real Time PCR, Pyrosequencing, High Resolution Melting PCR.

Several reports comparing the phenotyping and genotyping approach to predict DPD deficiency, reported conflicting results (89, 98-101). Coenen *et al.* provided an overview of 8 years of DPD testing in a single center (99), by using different methods, including radiochemical and non-radiochemical assay by ultra HPLC-MS in PBMCs with uracil, and a combined enzymatic and genetic test by Sanger sequence analysis of 4 DPYD variants. The analysis showed that 18% of patients with a genetic variant had decreased enzyme activity ($p < 0.001$), suggesting a combination of the genetic and the enzymatic test for diagnostic use. Similarly, Pallet *et al.*, comparing the phenotype and genotype in a pretherapeutic screening of DPD deficiency, observed that the use of UH2/U might better reflect the impact of genetic variants on DPD activity (101). A multicenter prospective cohort study assessed the clinical benefit of pretherapeutic screening for DPD deficiency using a multiparametric approach by the calculator 5-FUODPM Tox™ (89, 102). The pre-therapeutic DPD assessment reduced the incidence of early severe toxicities associated with 5-FU, and avoided early toxic death (100). Accordingly, Capitain *et al.* showed a comparison of 4 screening methods (genotyping, phenotyping via plasma U and plasma UH2/U, and a multi-parametric approach) for detecting 5-FU toxicity risk in 472 cancer patients. A lower false negative rate (4.7%) resulted from the multi-parametric methods ($p < 0.001$), compared to genotype (59.8%) and phenotype (36.1% and 21.3% for U and UH2/U, respectively), resulting as the most effective method for limiting G4-5 toxicity. Recently, Etienne-Grimaldi *et al.* showed that the combined phenotyping and genotyping approach increased sensitivity to both grade 3-4 toxicity (16.7% for genotyping versus 20.8% for the combined approach) and grade 4 toxicity (20% for genotyping versus 66.7% for the combined approach) (100).

Pre-treatment DPYD genotyping and clinical implementations

DPYD genotyping pre-treatment screening, and a dose reduction in patients carrying a deleterious variant, is a useful strategy to prevent severe and potentially lethal fluoropyrimidine-related toxicity, without diminishing the treatment efficacy. A reduced fluoropyrimidine dose of 50% in DPYD*2A carriers is the necessary strategy to prevent the risk of severe toxicity, as reported by Deenen *et al.* (103). Infact, fluoropyrimidine-induced mortality rate is reported as reduced from 10% to 0% by genotype-guided dose assessment (103). In a prospective, multicentre, safety analysis, 1.103 patients were screened for 4 different DPYD variants (DPYD*2A, DPYD*13, c.2846A > T, c.1236G > A), with a genotype-guided dose reduction of 25-50% based on DPYD genotype (50% for *2A or *13, 25% for c.2846A > T or c.1236G > A), improving patient safety with fluoropyrimidine treatment, and suggesting its genetic test implementation in routine clinical practice. As a result, the relative risk for severe fluoropyrimidine-related toxicity was lower in the cohort with the genotype-guided dosage compared to the historical cohort with no genotype-guided dosing. The authors also demonstrated a higher fluoropyrimidine-related severe toxicity in those patients carrying DPYD variants, than in wild-type patients (39% vs. 23%, $p = 0.0013$) (25). A small retrospective study successfully reported the implementation of routine pre-treatment DPYD genetic testing in patients with metastatic breast cancer treated with capecitabine (104). Seventy-two patients were eligible for capecitabine therapy and were tested for 4 DPYD genetic variants (DPYD*2A, DPYD*4, DPYD*13, c.2846A > T), based on their frequency in the British population, resulting associated with severe capecitabine-related toxicities. Five (8.4%) patients were found to carry a DPYD variant (*2A, *4 or c.2846A > T) associated with reduced DPD activity; of these, two received a 50% dose-reduction of capecitabine during their first cycle of treatment with no complications (104). A cohort of patients treated with fluoropyrimidine were tested for DPYD variants (DPYD*2A, DPYD*13, c.2846A > T, c.1236G > A) as part of routine practice. Two hundred and seventy-three (89.6%) out of 314 patients had a pre-treatment DPYD test result. Fourteen patients (5.1%) carried one or more DPYD gene variant and an initial dose reduction was recommended based on their genotype. None of these patients experienced severe toxicity (grade ≥ 3) (105).

The above-mentioned systematic meta-analyses (24, 40, 55) led researchers to evaluate the effective role of DPYD variants and their possibility to be screened as pre-treatment test in clinical routine. The Royal Dutch Association for the Advancement of Pharmacy's 'Pharmacogenetics Working Group' published a guideline supporting fluoropyrimidine-dose reduction for 14 DPYD variants (106). This guideline has been updated in a recent expert consensus guideline, by the Clinical Pharmacogenomics Implementation Consortium (CPIC), which reduced the number of variants to only those with robust supporting data (26). Accordingly, the Group of Clinical Pharmacology in Oncology (GPCO)-UNICANCER and the French Network of Pharmacogenetics (RN-PGx) (107), the German Society for Haematology and Medical Oncology (108), the Italian Association of Medical Oncology and the Italian Society of Pharmacology (AIOM-SIF working group) (109), and the Dutch Pharmacogenetics Working Group (DPWG) (110) published national recommendations or consensus papers for DPYD screening (**table I**). Recently, the European Medicines Agency (EMA) published a recommendation on DPD testing for patients prior to treatment with fluorouracil, capecitabine, tegafur (111), highlighting the importance of DPYD screening. Interestingly, a practitioner-friendly guide for clinicians to decide about DPYD genotyping testing prior to starting fluoropyrimidine-based chemotherapy has been published (112).

CONCLUSIONS

DPD-deficiency is an important leading cause of fluoropyrimidine-associated severe toxicity. Because of the extensive use of these anticancer agents, and the availability of feasible genotyping methods (*i.e.*, SNP genotyping by Real Time), the application of DPD variants screening has become easily accessible for clinical laboratories. To date, several consensus have been published concerning the clinical application of DPD screening in the population of patients candidates to fluoropyrimidine therapy, since patients carrying the deleterious DPYD*2A, DPYD*13 and c.2846T > C variant alleles display severe toxicities, which may be life-threatening in homozygous subjects. Although their frequencies are low, the screening for DPD variants is clinically relevant to avoid severe toxicities or death in patients treated with fluoropyrimidine-based chemotherapy. Future studies are necessary to

DPYD-GENOTYPING GUIDELINES	DPYD GENOTYPE	5-FU DOSE-REDUCTION SUGGESTED
CLINICAL PHARMACOGENOMICS IMPLEMENTATION CONSORTIUM (CPIC)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one no function allele or one decreased function allele (*1/2A; *1/13, *1/c.2846A > T, *1/c.1236G > A) or two decreased function allele (c.2846A > T/ c.2846A > T, c.1236G > A/ c.1236G > A)	25-50%
	Two decreased function alleles (*2A/*2A, *13/13) or one no function allele plus one decreased function allele (*2A/c.1236G > A, *2A/ c.2846A > T)	100%
GROUP OF CLINICAL PHARMACOLOGY IN ONCOLOGY (GPCO)-UNICANCER AND THE FRENCH NETWORK OF PHARMACOGENETICS (RNPGX)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one decreased function allele (*1/c.1236G > A or *1/c.2846A > T)	25-50%
	One normal function allele plus one no function allele (*1/*2A or *1/*13)	50%
	Two decreased function allele (*1/c.1236G > A and *1/c.2846A > T) or one reduced function allele plus one no function allele (combination of c.1236G > A or *1/c.2846A > T with *2A or *13, c.2846A > T)	Strongly reduced initial doses with drug monitoring
	Two no function alleles (*2A/*2A, *13/*13)	100%
ITALIAN ASSOCIATION OF MEDICAL ONCOLOGY AND THE ITALIAN SOCIETY OF PHARMACOLOGY (AIOM-SIF WORKING GROUP)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one decreased function allele (*1/c.1236G > A)	25%
	Patients who developed toxicity carrying *1/c.2194G > A alleles	15%
	One normal function allele plus one no function allele (*1/*2A, *1/*13 or *1/c.2846A > T)	50%
	Two decreased function alleles (c.1236G > A)	50%
	Two no function alleles (*2A/*2A, *13/*13, c.2846A > T)	100%
	Patients who developed toxicity carrying two decreased function alleles (c.2194G > A)	30%
DUTCH PHARMACOGENETICS WORKING GROUP (DPWG)	Two normal function alleles (*1/*1)	0%
	One normal function allele plus one decreased function allele (*1/c.1236G > A or *1/c.2846A > T)	25%
	One normal function allele plus one no function allele (*1/*2A or *1/*13)	50%
	Two decreased function alleles (*2A/*2A, *13/*13)	100%

Table I. DPYD-genotyping guidelines.

highlight the correlation with toxicity of other DPYD SNPs, including the c.2194G > A variant, for which interesting data have been recently reported.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

1. Miura K, Kinouchi M, Ishida K, et al. 5-fu metabolism in cancer and orally-administrable 5-fu drugs. *Cancers (Basel)* 2010;2(3):1717-30.
2. Cameron DA, Gabra H, Leonard RC. Continuous 5-fluorouracil in the treatment of breast cancer. *Br J Cancer* 1994;70(1):120-4.
3. Vodenkova S, Buchler T, Cervena K, Veskrnova V, Vodicka P, Vymetalkova V. 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. *Pharmacol Ther* 2020;206:107447.
4. Fu KK. Radiation Therapy With 5-fluorouracil in Head and Neck Cancer. *Semin Radiat Oncol* 1997;7(4):274-82.
5. Van Cutsem E, Peeters M. Developments in fluoropyrimidine therapy for gastrointestinal cancer. *Curr Opin Oncol* 1999;11(4):312-7.
6. Conroy T, Desseigne F, Ychou M, et al. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011;364(19):1817-25.
7. Fischel JL, Rostagno P, Formento P, Dubreuil A, Etienne MC, Milano G. Ternary combination of irinotecan, fluorouracil-folinic acid and oxalipatin: results on human colon cancer cell lines. *Br J Cancer* 2001;84(4):579-85.
8. Periti P, Pannuti F, Della Cuna GR, et al. Combination chemotherapy with cyclophosphamide, fluorouracil, and either epirubicin or mitoxantrone: a comparative randomized multicenter study in metastatic breast carcinoma. *Cancer Invest* 1991;9(3):249-55.
9. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350(23):2335-42.
10. Min BS, Kim NK, Ahn JB, et al. Cetuximab in combination with 5-fluorouracil, leucovorin and irinotecan as a neoadjuvant chemotherapy in patients with initially unresectable colorectal liver metastases. *Onkologie* 2007;30(12):637-43.
11. Wigmore PM, Mustafa S, El-Beltagy M, Lyons L, Umka J, Bennett G. Effects of 5-FU. *Adv Exp Med Biol* 2010;678:157-64.
12. Han R, Yang YM, Dietrich J, Luebke A, Mayer-Proschel M, Noble M. Systemic 5-fluorouracil treatment causes a syndrome of delayed myelin destruction in the central nervous system. *J Biol* 2008;7(4):12.
13. Walko CM, Lindley C. Capecitabine: a review. *Clin Ther* 2005;27(1):23-44.
14. McGavin JK, Goa KL. Capecitabine: a review of its use in the treatment of advanced or metastatic colorectal cancer. *Drugs* 2001;61(15):2309-26.
15. Boisdron-Celle M, Morel A, Gamelin E. Dihydropyrimidine dehydrogenase deficiency and toxicity to fluoropyrimidine. *Ann Biol Clin (Paris)* 2010;68(1):27-32.
16. Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003;3(5):330-8.
17. van Kuilenburg AB, Meinsma R, Zonnenberg BA, et al. Dihydropyrimidinase deficiency and severe 5-fluorouracil toxicity. *Clin Cancer Res* 2003;9(12):4363-7.
18. Houghton JA, Morton CL, Adkins DA, Rahman A. Locus of the interaction among 5-fluorouracil, leucovorin, and interferon-alpha 2a in colon carcinoma cells. *Cancer Res* 1993;53(18):4243-50.
19. Noordhuis P, Holwerda U, Van der Wilt CL, et al. 5-Fluorouracil incorporation into RNA and DNA in relation to thymidylate synthase inhibition of human colorectal cancers. *Ann Oncol* 2004;15(7):1025-32.
20. Omura K. Clinical implications of dihydropyrimidine dehydrogenase (DPD) activity in 5-FU-based chemotherapy: mutations in the DPD gene, and DPD inhibitory fluoropyrimidines. *Int J Clin Oncol* 2003;8(3):132-8.
21. Gross E, Busse B, Riemenschneider M, et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. *PLoS One* 2008;3(12):e4003.
22. Wei X, Elizondo G, Sapone A, et al. Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics* 1998;51(3):391-400.
23. Takai S, Fernandez-Salguero P, Kimura S, Gonzalez FJ, Yamada K. Assignment of the human dihydropyrimidine dehydrogenase gene (DPYD) to chromosome region 1p22 by fluorescence in situ hybridization. *Genomics* 1994;24(3):613-4.
24. Meulendijks D, Henricks LM, Sonke GS, et al. Clinical relevance of DPYD variants c.1679T > G, c.1236G > A/HapB3, and c.1601G > A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data. *Lancet Oncol* 2015;16(16):1639-50.

25. Henricks LM, Lunenburg C, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol* 2018;19(11):1459-67.
26. Amstutz U, Henricks LM, Offer SM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. *Clin Pharmacol Ther* 2018;103(2):210-6.
27. Del Re M, Cinieri S, Michelucci A, et al. DPYD*6 plays an important role in fluoropyrimidine toxicity in addition to DPYD*2A and c.2846A > T: a comprehensive analysis in 1254 patients. *Pharmacogenomics J* 2019;19(6):556-63.
28. Etienne MC, Lagrange JL, Dassonville O, et al. Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol* 1994;12(11):2248-53.
29. Lu Z, Zhang R, Carpenter JT, Diasio RB. Decreased dihydropyrimidine dehydrogenase activity in a population of patients with breast cancer: implication for 5-fluorouracil-based chemotherapy. *Clin Cancer Res* 1998;4(2):325-9.
30. Ogura K, Ohnuma T, Minamide Y, et al. Dihydropyrimidine dehydrogenase activity in 150 healthy Japanese volunteers and identification of novel mutations. *Clin Cancer Res* 2005;11(14):5104-11.
31. Yamaguchi K, Arai Y, Kanda Y, Akagi K. Germline mutation of dihydropyrimidine dehydrogenase gene among a Japanese population in relation to toxicity to 5-Fluorouracil. *Jpn J Cancer Res* 2001;92(3):337-42.
32. Saif MW, Mattison L, Carollo T, Ezzeldin H, Diasio RB. Dihydropyrimidine dehydrogenase deficiency in an Indian population. *Cancer Chemother Pharmacol* 2006;58(3):396-401.
33. Hamdy SI, Hiratsuka M, Narahara K, et al. Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol* 2002;53(6):596-603.
34. Seck K, Riemer S, Kates R, et al. Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in a cohort of Caucasian individuals. *Clin Cancer Res* 2005;11(16):5886-92.
35. Van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. Increased risk of grade IV neutropenia after administration of 5-fluorouracil due to a dihydropyrimidine dehydrogenase deficiency: high prevalence of the IVS14 + 1g > a mutation. *Int J Cancer* 2002;101(3):253-8.
36. Offer SM, Wegner NJ, Fossum C, Wang K, Diasio RB. Phenotypic profiling of DPYD variations relevant to 5-fluorouracil sensitivity using real-time cellular analysis and in vitro measurement of enzyme activity. *Cancer Res* 2013;73(6):1958-68.
37. Offer SM, Fossum CC, Wegner NJ, Stufless AJ, Butterfield GL, Diasio RB. Comparative functional analysis of DPYD variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. *Cancer Res* 2014;74(9):2545-54.
38. Lee AM, Shi Q, Pavey E, et al. DPYD variants as predictors of 5-fluorouracil toxicity in adjuvant colon cancer treatment (NCCTG N0147). *J Natl Cancer Inst* 2014;106(12).
39. van Kuilenburg AB, Muller EW, Haasjes J, et al. Lethal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency after administration of 5-fluorouracil: frequency of the common IVS14 + 1G > A mutation causing DPD deficiency. *Clin Cancer Res* 2001;7(5):1149-53.
40. Terrazzino S, Cargnin S, Del Re M, Danesi R, Canonico PL, Genazzani AA. DPYD IVS14 + 1G > A and 2846A > T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. *Pharmacogenomics* 2013;14(11):1255-72.
41. van Kuilenburg AB, Dobritzsch D, Meinsma R, Haasjes J, Waterham HR, Nowaczyk MJ, et al. Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. *Biochem J* 2002;364(Pt 1):157-63.
42. Caudle KE, Thorn CF, Klein TE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. *Clin Pharmacol Ther* 2013;94(6):640-5.
43. Meulendijks D, Henricks LM, van Kuilenburg AB, et al. Patients homozygous for DPYD c.1129-5923C > G/haplotype B3 have partial DPD deficiency and require a dose reduction when treated with fluoropyrimidines. *Cancer Chemother Pharmacol* 2016;78(4):875-80.
44. van Kuilenburg AB, Meijer J, Mul AN, et al. Intragenic deletions and a deep intronic mutation affecting pre-mRNA splicing in the dihy-

- dropyrimidine dehydrogenase gene as novel mechanisms causing 5-fluorouracil toxicity. *Hum Genet* 2010;128(5):529-38.
45. Amstutz U, Farese S, Aebi S, Largiader CR. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics* 2009;10(6):931-44.
 46. Deenen MJ, Tol J, Burylo AM, et al. Relationship between single nucleotide polymorphisms and haplotypes in DPYD and toxicity and efficacy of capecitabine in advanced colorectal cancer. *Clin Cancer Res* 2011;17(10):3455-68.
 47. Sistonen J, Buchel B, Froehlich TK, et al. Predicting 5-fluorouracil toxicity: DPD genotype and 5,6-dihydrouracil:uracil ratio. *Pharmacogenomics* 2014;15(13):1653-66.
 48. Ridge SA, Sludden J, Brown O, et al. Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *Br J Clin Pharmacol* 1998;46(2):151-6.
 49. Maekawa K, Saeki M, Saito Y, et al. Genetic variations and haplotype structures of the DPYD gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences. *J Hum Genet* 2007;52(10):804-19.
 50. He YF, Wei W, Zhang X, et al. Analysis of the DPYD gene implicated in 5-fluorouracil catabolism in Chinese cancer patients. *J Clin Pharm Ther* 2008;33(3):307-14.
 51. Offer SM, Lee AM, Mattison LK, Fossum C, Wegner NJ, Diasio RB. A DPYD variant (Y186C) in individuals of african ancestry is associated with reduced DPD enzyme activity. *Clin Pharmacol Ther* 2013;94(1):158-66.
 52. Kleibl Z, Fidlerova J, Kleiblova P, et al. Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine-related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine-based chemotherapy. *Neoplasma* 2009;56(4):303-16.
 53. Saif MW, Diasio R. Is capecitabine safe in patients with gastrointestinal cancer and dihydropyrimidine dehydrogenase deficiency? *Clin Colorectal Cancer* 2006;5(5):359-62.
 54. van Kuilenburg AB, Largiader CR. SNPs and Haplotypes in DPYD and Outcome of Capecitabine-Letter. *Clin Cancer Res* 2011;17(17):5837; author reply 5-6.
 55. Rosmarin D, Palles C, Church D, et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. *J Clin Oncol* 2014;32(10):1031-9.
 56. Gbeto CC, Quaranta S, Mari R, et al. Lethal toxicities after capecitabine intake in a previously 5-FU-treated patient: why dose matters with dihydropyrimidine dehydrogenase deficiency. *Pharmacogenomics* 2019;20(13):931-8.
 57. Shakeel F, Fang F, Kwon JW, et al. Patients carrying DPYD variant alleles have increased risk of severe toxicity and related treatment modifications during fluoropyrimidine chemotherapy. *Pharmacogenomics* 2021.
 58. Toffoli G, Giodini L, Buonadonna A, et al. Clinical validity of a DPYD-based pharmacogenetic test to predict severe toxicity to fluoropyrimidines. *Int J Cancer* 2015;137(12):2971-80.
 59. Ruzzo A, Graziano F, Galli F, et al. Dihydropyrimidine dehydrogenase pharmacogenetics for predicting fluoropyrimidine-related toxicity in the randomised, phase III adjuvant TOSCA trial in high-risk colon cancer patients. *Br J Cancer* 2017;117(9):1269-77.
 60. Boige V, Vincent M, Alexandre P, et al. DPYD Genotyping to Predict Adverse Events Following Treatment With Fluorouracil-Based Adjuvant Chemotherapy in Patients With Stage III Colon Cancer: A Secondary Analysis of the PETACC-8 Randomized Clinical Trial. *JAMA Oncol* 2016;2(5):655-62.
 61. Gross E, Ullrich T, Seck K, et al. Detailed analysis of five mutations in dihydropyrimidine dehydrogenase detected in cancer patients with 5-fluorouracil-related side effects. *Hum Mutat* 2003;22(6):498.
 62. Vreken P, Van Kuilenburg AB, Meinsma R, van Gennip AH. Dihydropyrimidine dehydrogenase (DPD) deficiency: identification and expression of missense mutations C29R, R886H and R235W. *Hum Genet* 1997;101(3):333-8.
 63. Khushman M, Patel GK, Hosein PJ, et al. Germline pharmacogenomics of DPYD*9A (c.85T > C) variant in patients with gastrointestinal malignancies treated with fluoropyrimidines. *J Gastrointest Oncol* 2018;9(3):416-24.
 64. Dettailleur S, Segelov E, Re MD, Prenen H. Dihydropyrimidine dehydrogenase deficiency in patients with severe toxicity after 5-fluorouracil: a retrospective single-center study. *Ann Gastroenterol* 2021;34(1):68-72.
 65. Zhang H, Li YM, Zhang H, Jin X. DPYD*5 gene

- mutation contributes to the reduced DPYD enzyme activity and chemotherapeutic toxicity of 5-FU: results from genotyping study on 75 gastric carcinoma and colon carcinoma patients. *Med Oncol* 2007;24(2):251-8.
66. Boisdron-Celle M, Remaud G, Traore S, et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. *Cancer Lett* 2007;249(2):271-82.
 67. Morel A, Boisdron-Celle M, Fey L, et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Mol Cancer Ther* 2006;5(11):2895-904.
 68. Van Kuilenburg AB, Vreken P, Abeling NG, et al. Genotype and phenotype in patients with dihydropyrimidine dehydrogenase deficiency. *Hum Genet* 1999;104(1):1-9.
 69. Hsiao HH, Yang MY, Chang JG, et al. Dihydropyrimidine dehydrogenase pharmacogenetics in the Taiwanese population. *Cancer Chemother Pharmacol* 2004;53(5):445-51.
 70. Lazar A, Mau-Holzmann UA, Kolb H, Reichenmiller HE, Riess O, Schomig E. Multiple organ failure due to 5-fluorouracil chemotherapy in a patient with a rare dihydropyrimidine dehydrogenase gene variant. *Onkologie* 2004;27(6):559-62.
 71. Cunha-Junior GF, Bastos-Rodrigues L, Azevedo PG, et al. Prevalence of the DPYD variant (Y186C) in Brazilian individuals of African ancestry. *Cancer Chemother Pharmacol* 2019;84(6):1359-63.
 72. Teh LK, Hamzah S, Hashim H, et al. Potential of dihydropyrimidine dehydrogenase genotypes in personalizing 5-fluorouracil therapy among colorectal cancer patients. *Ther Drug Monit* 2013;35(5):624-30.
 73. Li GY, Duan JF, Li WJ, Liu T. DPYD*2A/*5A/*9A and UGT1A1*6/*28 polymorphisms in Chinese colorectal cancer patients. *J Cancer Res Ther* 2016;12(2):782-6.
 74. Hariprakash JM, Vellarikkal SK, Keechilat P, et al. Pharmacogenetic landscape of DPYD variants in south Asian populations by integration of genome-scale data. *Pharmacogenomics* 2018;19(3):227-41.
 75. Del Re M, Qua Quarini E, Sottotetti F, et al. Uncommon dihydropyrimidine dehydrogenase mutations and toxicity by fluoropyrimidines: a lethal case with a new variant. *Pharmacogenomics* 2016;17(1):5-9.
 76. Del Re M, Michelucci A, Di Leo A, et al. Discovery of novel mutations in the dihydropyrimidine dehydrogenase gene associated with toxicity of fluoropyrimidines and viewpoint on preemptive pharmacogenetic screening in patients. *EPMA J* 2015;6(1):17.
 77. Palmirotta R, Lovero D, Delacour H, Le Roy A, Cremades S, Silvestris F. Rare Dihydropyrimidine Dehydrogenase Variants and Toxicity by Fluoropyrimidines: A Case Report. *Front Oncol* 2019;9:139.
 78. Hamzic S, Scharer D, Offer S, et al. Haplotype structure defines effects of common DPYD variants c.85T > C (rs1801265) and c.496A > G (rs2297595) on DPD activity: implication for 5-fluorouracil toxicity. *Br J Clin Pharmacol* 2021.
 79. Falvella FS, Cheli S, Martinetti A, et al. DPD and UGT1A1 deficiency in colorectal cancer patients receiving triplet chemotherapy with fluoropyrimidines, oxaliplatin and irinotecan. *Br J Clin Pharmacol* 2015;80(3):581-8.
 80. Lee AM, Shi Q, Alberts SR, et al. Association between DPYD c.1129-5923 C > G/hapB3 and severe toxicity to 5-fluorouracil-based chemotherapy in stage III colon cancer patients: NCCTG N0147 (Alliance). *Pharmacogenet Genomics* 2016;26(3):133-7.
 81. Zhang XP, Bai ZB, Chen BA, et al. Polymorphisms of dihydropyrimidine dehydrogenase gene and clinical outcomes of gastric cancer patients treated with fluorouracil-based adjuvant chemotherapy in Chinese population. *Chin Med J (Engl)* 2012;125(5):741-6.
 82. van Kuilenburg AB, Meijer J, Maurer D, et al. Severe fluoropyrimidine toxicity due to novel and rare DPYD missense mutations, deletion and genomic amplification affecting DPD activity and mRNA splicing. *Biochim Biophys Acta Mol Basis Dis* 2017;1863(3):721-30.
 83. Shrestha S, Tapper EE, Trogstad-Isaacson CS, Hobday TJ, Offer SM, Diasio RB. Dose modification for safe treatment of a compound complex heterozygous DPYD variant carrier with 5-fluorouracil. *JCO Precis Oncol* 2018;2.
 84. Tong CC, Lam CW, Lam KO, Lee VHF, Luk MY. A Novel DPYD Variant Associated With Severe Toxicity of Fluoropyrimidines: Role of Pre-emptive DPYD Genotype Screening. *Front Oncol* 2018;8:279.
 85. Garcia-Gonzalez X, Kaczmarczyk B, Abarca-Zabalia J, et al. New DPYD variants causing DPD deficiency in patients treated with fluoropyrimidine. *Cancer Chemother Pharmacol* 2020;86(1):45-54.

86. van Kuilenburg AB, Baars JW, Meinsma R, van Gennip AH. Lethal 5-fluorouracil toxicity associated with a novel mutation in the dihydropyrimidine dehydrogenase gene. *Ann Oncol* 2003;14(2):341-2.
87. Shrestha S, Zhang C, Jerde CR, et al. Gene-Specific Variant Classifier (DPYD-Varifier) to Identify Deleterious Alleles of Dihydropyrimidine Dehydrogenase. *Clin Pharmacol Ther* 2018;104(4):709-18.
88. Ly RC, Schmidt RE, Kiel PJ, et al. Severe Capecitabine Toxicity Associated With a Rare DPYD Variant Identified Through Whole-Genome Sequencing. *JCO Precis Oncol* 2020;4.
89. Boisdron-Celle M, Capitain O, Faroux R, et al. Prevention of 5-fluorouracil-induced early severe toxicity by pre-therapeutic dihydropyrimidine dehydrogenase deficiency screening: Assessment of a multiparametric approach. *Semin Oncol* 2017;44(1):13-23.
90. Bocci G, Barbara C, Vannozzi F, et al. A pharmacokinetic-based test to prevent severe 5-fluorouracil toxicity. *Clin Pharmacol Ther* 2006;80(4):384-95.
91. Ciccolini J, Mercier C, Evrard A, et al. A rapid and inexpensive method for anticipating severe toxicity to fluorouracil and fluorouracil-based chemotherapy. *Ther Drug Monit* 2006;28(5):678-85.
92. Wettergren Y, Carlsson G, Odin E, Gustavsson B. Pretherapeutic uracil and dihydrouracil levels of colorectal cancer patients are associated with sex and toxic side effects during adjuvant 5-fluorouracil-based chemotherapy. *Cancer* 2012;118(11):2935-43.
93. Meulendijks D, Henricks LM, Jacobs BAW, et al. Pretreatment serum uracil concentration as a predictor of severe and fatal fluoropyrimidine-associated toxicity. *Br J Cancer* 2017;116(11):1415-24.
94. van Staveren MC, van Kuilenburg AB, Guchelaar HJ, et al. Evaluation of an oral uracil loading test to identify DPD-deficient patients using a limited sampling strategy. *Br J Clin Pharmacol* 2016;81(3):553-61.
95. Di Paolo A, Danesi R, Falcone A, et al. Relationship between 5-fluorouracil disposition, toxicity and dihydropyrimidine dehydrogenase activity in cancer patients. *Ann Oncol* 2001;12(9):1301-6.
96. Van Kuilenburg AB, Van Lenthe H, Van Gennip AH. Radiochemical assay for determination of dihydropyrimidine activity using reversed-phase high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1999;729(1-2):307-14.
97. Van Kuilenburg AB, van Lenthe H, Blom MJ, Mul EP, Van Gennip AH. Profound variation in dihydropyrimidine dehydrogenase activity in human blood cells: major implications for the detection of partly deficient patients. *Br J Cancer* 1999;79(3-4):620-6.
98. Capitain O, Seegers V, Metges JP, et al. Comparison of 4 Screening Methods for Detecting Fluoropyrimidine Toxicity Risk: Identification of the Most Effective, Cost-Efficient Method to Save Lives. *Dose Response* 2020;18(3):1559325820951367.
99. Coenen MJH, Paulussen ADC, Breuer M, et al. Evolution of Dihydropyrimidine Dehydrogenase Diagnostic Testing in a Single Center during an 8-Year Period of Time. *Curr Ther Res Clin Exp* 2019;90:1-7.
100. Etienne-Grimaldi MC, Boyer JC, Beroud C, et al. New advances in DPYD genotype and risk of severe toxicity under capecitabine. *PLoS One* 2017;12(5):e0175998.
101. Pallet N, Hamdane S, Garinet S, et al. A comprehensive population-based study comparing the phenotype and genotype in a pretherapeutic screen of dihydropyrimidine dehydrogenase deficiency. *Br J Cancer* 2020;123(5):811-8.
102. Available at: <http://www.odpm.fr>. Accessed: 04/02/2021.
103. Deenen MJ, Meulendijks D, Cats A, et al. Up-front Genotyping of DPYD*2A to Individualize Fluoropyrimidine Therapy: A Safety and Cost Analysis. *J Clin Oncol* 2016;34(3):227-34.
104. Stavrika C, Pouptsis A, Okonta L, et al. Clinical implementation of pre-treatment DPYD genotyping in capecitabine-treated metastatic breast cancer patients. *Breast Cancer Res Treat* 2019;175(2):511-7.
105. Lunenburg C, Henricks LM, Guchelaar HJ, et al. Prospective DPYD genotyping to reduce the risk of fluoropyrimidine-induced severe toxicity: Ready for prime time. *Eur J Cancer* 2016;54:40-8.
106. Swen JJ, Nijenhuis M, de Boer A, et al. Pharmacogenetics: from bench to byte--an update of guidelines. *Clin Pharmacol Ther* 2011;89(5):662-73.
107. Lorient MA, Ciccolini J, Thomas F, et al. Dihydropy-

- rimidinedehydrogenase(DPD)deficiencyscreening and securing of fluoropyrimidine-based chemotherapies: Update and recommendations of the French GPCO-Unicancer and RNPgX networks. *Bull Cancer* 2018;105(4):397-407.
108. Wormann B, Bokemeyer C, Burmeister T, et al. Dihydropyrimidine Dehydrogenase Testing prior to Treatment with 5-Fluorouracil, Capecitabine, and Tegafur: A Consensus Paper. *Oncol Res Treat* 2020;43(11):628-36.
 109. AIOM-SIF Working group. Recommendations 2019for pharmacogenetic analyses, 2019-Available at: https://www.aiom.it/wp-content/uploads/2019/10/2019_Racc-analisi-farmacogenetiche_v26.3.2020.pdf. Accessed:10/01/2021.
 110. Lunenburg C, van der Wouden CH, Nijenhuis M, et al. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of DPYD and fluoropyrimidines. *Eur J Hum Genet* 2020;28(4):508-17.
 111. EMA/367286/2020 (2020). EMA recommendations on DPD testing prior to treatment with fluorouracil, capecitabine, tegafur and flucytosine. Available at: https://www.ema.europa.eu/en/documents/referral/fluorouracil-fluorouracil-related-substances-article-31-referral-new-testing-treatment_en.pdf. Accessed: 10/01/2021.
 112. Innocenti F, Mills SC, Sanoff H, Ciccolini J, Lenz HJ, Milano G. All You Need to Know About DPYD Genetic Testing for Patients Treated With Fluorouracil and Capecitabine: A Practitioner-Friendly Guide. *JCO Oncol Pract* 2020;16(12):793-8.

REVIEW

COMBINATION IMMUNOTHERAPY IN METASTATIC RENAL CELL CARCINOMA

P. Sepe, A. Ottini, E. Verzoni, V. Guadalupi, M. Claps, G. Procopio

Department of Medical Oncology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy

CORRESPONDING AUTHOR:

Pierangela Sepe
Department of Medical Oncology Unit
Fondazione IRCCS Istituto Nazionale dei Tumori di Milano
via G. Venezian 1
20133 Milan, Italy
E-mail: pierangela.sepe@istitutotumori.mi.it

Doi: 10.48286/aor.2021.10

ABSTRACT

Various treatments have been considered as the cornerstone for the management of patients with metastatic renal cell carcinoma (mRCC) over the past two decades. Currently, immunotherapy is a promising clue in the landscape of frontline treatment of mRCC. Immune checkpoint inhibitors (ICIs), which constitute a standard therapy in pre-treated mRCC patients, are emerging as possible earlier treatment's strategy in mRCC. Otherwise, antiangiogenetics are well established as a backbone therapy for mRCC, and research is now focused on development of innovative tyrosine kinase inhibitors (TKIs). Frontline combination with ICIs as well as strategies including both TKIs and immunotherapy demonstrated to significantly improve outcomes compared to single-agent an-

tiangiogenetics. Nonetheless, a considerable proportion of patients shows primary resistance to ICIs and new approaches are currently emerging to resolve this important unmet need. Moreover, several treatment strategies combining different mechanisms of action or targeting immune escape pathways are rising with the objective of improving response rates and patient's outcomes. This review summarizes current immunotherapeutic agents approved for mRCC.

KEY WORDS

Metastatic renal cell carcinoma; immunotherapy; anti-PD-1; anti-CTLA-4; targeted therapy; biomarkers.

INTRODUCTION

Kidney cancer has peculiar features that make it attractive for immunotherapeutic approaches: chemoresistance and immunogenicity (1). Angiogenesis and immunosuppression play a relevant role in metastatic renal cell carcinoma (mRCC) car-

cinogenesis. Over the last two decades, different therapies including angiogenesis inhibitor monoclonal antibodies, multitarget molecules such as vascular endothelial growth factor receptor (VEGFR) inhibitors, and other tyrosine kinase inhibitors (TKIs), as well mammalian target of rapamycin (mTOR) in-

hibitors, have been considered the backbone for the treatment of mRCC (2-7). On the other hand, a new therapeutic approach has opened up in the frontline setting of treatment with immunotherapy (8, 9).

Immune checkpoint inhibitors (ICI's) already represent a well know treatment option in pretreated mRCC patients, and combination immunotherapy as well as combinations of immunotherapy with targeted agents shown to significantly improve the outcomes of treatment-naïve mRCC patients (10-16). In mRCC, immunotherapy enhances adaptive immunity, granting the possibility for the immune system to recognize tumor antigens and to kill malignant cells (17). Activation of adaptive immunity against neoplastic antigens involves different receptors present on both malignant cells and immune cells, with inhibition or activation of signals (17). The tangled biological pathway underlying the role of anticancer immunity and its manipulation with therapies is not yet fully established. The immune compartment mainly includes T cells, natural-killer (NK) cells, B cells, macrophages and dendritic cells with complex interactions. T-cells are probably the major factor for both cellular and humoral immunological control of tumor growth. CD8+ T- cells are the main effectors of the anti-tumor immune response, recognizing antigens expressed by malignant cells and, once activated, killing neoplastic cells (18). CD4+ T-cells help in generating an immune response by stimulating CD8+ T-cells, macrophages and B-lymphocytes. The activation of effector and memory CD8+ T-cells occurs by the interaction with antigen-presenting cells (APC) via the T-cell receptor (TCR) and major histocompatibility complex (MHC)/peptide antigen (19). The most known ICIs are the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) pathway and the Programmed death 1 (PD-1) with its ligand (PD-L1) (17, 20). These two complexes are the targets of several drugs experimented in different clinical trials and now included in clinical practice. Anti CTLA-4 agents modulate the activation of T-cells binding to the CD80 and CD86 ligands, compromising CD28 for greater affinity (21). This results in a modulated activation of naïve T-cells and memory. The success achieved by ipilimumab, a CTLA-4 inhibitor, in metastatic melanoma demonstrated that the inhibition of this immuno-checkpoint might stimulate the host immune system against neoplastic antigens, with tumor cells' death (22). However, in mRCC ipilimumab did not show a similar significative benefit. PD-1 is an inhibitory receptor expressed by activated T-cells, B-cells, monocytes, and natural killer (NK) cells. PD-

L1 and PD-L2 are two well-known ligands that activate PD-1. In particular, PD-L1 is expressed in different cells, including APC and malignant cells. The interplay of PD-1 with PD-L1 is responsible for the immunosuppressive effects of PD-1 (23) by inhibiting the proliferation, survival, and function of CD8+ lymphocytes, and promoting the differentiation of CD4+ T-cells into regulatory T lymphocytes (Tregs). This mechanism can induce apoptosis of infiltrating tumor cells. Nonetheless, PD-L2 is responsible for the inhibition of T-cell activation (23). Anti-PD1 agents, such as pembrolizumab and nivolumab, and anti-PD-L1 drugs, such as durvalumab, atezolizumab and avelumab, led to a revolutionary approach in the therapeutic management of different solid neoplasms, including melanoma, non-small cell lung cancer, mRCC, urothelial carcinoma and Merkel cell carcinoma, but also in hematologic malignancies such as lymphomas (10-15, 24). This review summarizes current immunotherapeutic agents approved for mRCC.

Combination therapy

The frontline treatment of mRCC has been transformed since the approval of the immunotherapeutic combination. The two regulatory agency Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved in 2018 the combination nivolumab plus ipilimumab in treatment-naïve mRCC patients at intermediate-poor risk and two other combinations in 2019 regardless of the risk category group: avelumab plus axitinib and pembrolizumab plus axitinib.

Immune Checkpoint Inhibitors

In the Checkmate 214 phase III trial 1096 treatment-naïve mRCC patients were randomly assigned to receive nivolumab 3 mg/kg plus ipilimumab 1 mg/kg every 3 weeks, followed by nivolumab at the same dose every 2 weeks *versus* sunitinib 50 mg daily schedule 4 weeks on and 2 weeks off. Primary endpoints were objective response rate (ORR), progression-free survival (PFS) and overall survival (OS) in International mRCC Database Consortium (IMDC) intermediate-poor risk patients. Secondary endpoints were ORR, PFS and OS in any risk patients and safety in all treated patients. ORR, PFS and OS in IMDC favorable-risk were exploratory endpoints. At the first data cut-off, OS, PFS and ORR resulted significantly improved for the combination nivolumab plus ipilimumab *versus* sunitinib in intermediate-poor-risk patients (11).

TRIAL	TREATMENT ARM VS COMPARISON ARM	SETTING	ENDPOINT	MOS (MONTHS) ^a HR (95% CI) P	MPFS (MONTHS) ^a HR (95% CI) P	ORR ^a P	GRADE 3 AND 4 TRAEs ^a
CHECKMATE 214	Nivolumab ^b 3 mg/kg i.v. + Ipilimumab ^b 1 mg/kg i.v. vs Sunitinib 50 mg orally OD 4w on/2w off	First line, intermediate- or poor-risk mRCC	Primary: OS; PFS; ORR in intermediate or poor-risk patients Secondary: OS; PFS; ORR in ITT; safety	Intermediate or poor-risk patients: 47 vs 26.6 HR 0.66; (0.55-0.80) p < 0.0001 ITT: HR 0.72; (0.61-0.86) p = 0.0002	Intermediate or poor- risk patients: 12 vs 8.3 HR 0.76; (0.63-0.91) p < 0.01 ITT: HR 0.89; (0.76-1.05) p = 0.02	Intermediate or poor-risk patients: 42% vs 26% p = 0.0001 ITT: 39% vs 33% p = 0.02	47% vs 64%
JAVELIN RENAL 101	Avelumab 10 mg/kg i.v. q2w + Axitinib 5 mg orally BID vs Sunitinib 50 mg Orally OD 4w on/2w off	First line mRCC	Primary: PFS and OS among PD-L1+ patients Secondary: OS and PFS in the overall population	PD-L1 + NR vs NR HR 0.828 (0.596-1.151) one-sided p = 0.1301 Overall population NR vs NR HR 0.796 (0.616-1.027); one-sided p = 0.0392	PD-L1 + 13.8 vs 7 HR 0.62 (0.490-0.777) p < 0.0001 Overall population 13.3 vs 8 0.69 (0.574-0.825) p < 0.0001	51.4% vs 25.7 % 71.2% vs. 71.5%	
KEYNOTE 426	Pembrolizumab 200 mg i.v. q3w + Axitinib 5 mg orally BID vs Sunitinib 50 mg Orally OD 4w on/2w off	First line mRCC	Primary: OS; PFS Secondary: ORR; DOR; safety	NR vs 35.7 HR 0.68; (0.55-0.85) p < 0.001	15.4 vs 11.1 HR 0.71 (0.60-0.84) p < 0.001	60% vs 40% p < 0.0001	62.9% vs 58.1%
CHECKMATE 9ER	Nivolumab 240 mg i.v. q3w + Cabozantinib 40 mg orally vs Sunitinib 50 mg Orally OD 4w on/2w off	First line mRCC	Primary: PFS Secondary: OS, ORR; safety	NR	16.6 vs 8.3 months HR 0.51; (0.41-0.64) P = 0.0001	55.7% vs 27.1% p < 0.0001	61% vs 51

Table 1. Immune checkpoint inhibitors in first line for metastatic renal cell carcinoma.

^aExperimental arm vs standard of care arm.

^bNivolumab (3 mg/kg) plus ipilimumab (1 mg/kg) intravenously every 3 weeks for four doses, followed by nivolumab (3 mg/kg) every 2 weeks.

List of terms: BID: bis in die; CI, confidence interval; DOR, duration of response, HR, hazard ratio; kg, kilogram; ITT, intention-to-treat population; i.v., intravenous; mg, milligram; mRCC, Metastatic Renal Cell Carcinoma; (m)OS, (median) overall survival; (m)PFS, (median) progression-free survival; NR, not reached; OD: once daily; ORR, objective response rate; PD-L1: Programmed Death 1, TRAEs Treatment-related adverse events, independent radiology review committee; VS: versus; w = week.

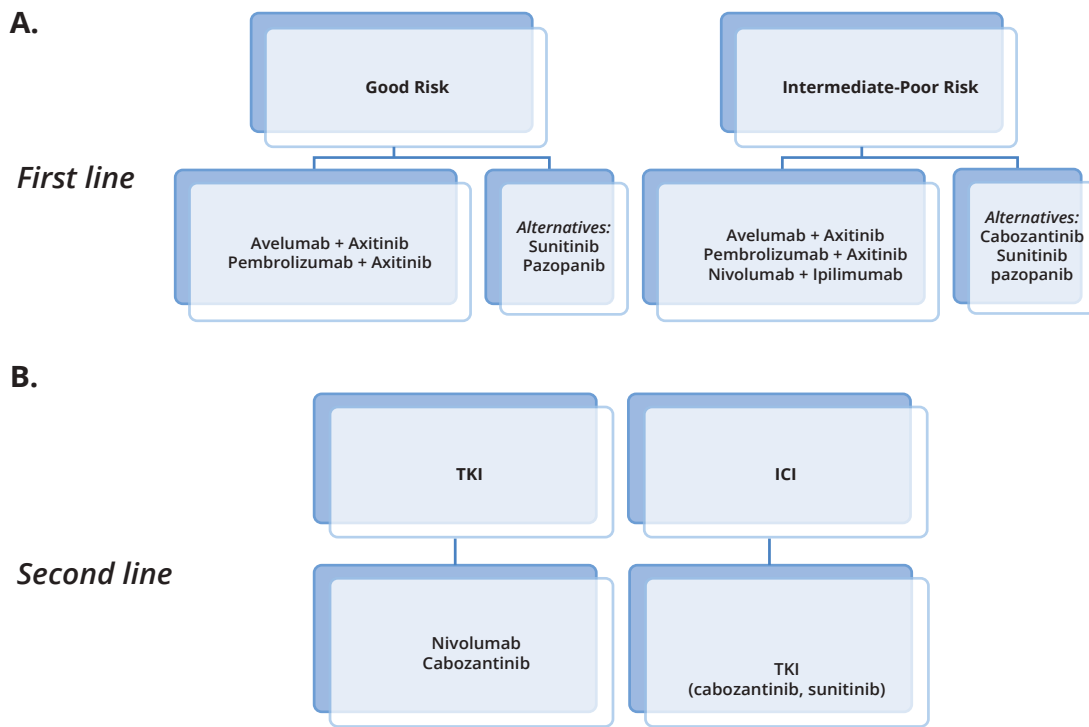


Figure 1. a. Summary of immunotherapy combinations approved by EMA and FDA for the first line treatment of metastatic renal cell carcinoma according to risk category group. TKI could also be an option; **b.** Panel of options for the second lines after ICI or TKI. Abbreviations: TKI, tyrosine kinase inhibitor; ICI, Immune checkpoint inhibitor.

The significant superiority of the immunotherapeutic combination over sunitinib for intermediate-poor risk patients with mRCC was confirmed at the median follow-up of 42 months. In summary, long-term results showed the maintained benefit for the combination over sunitinib in terms of OS (47.0 *versus* 26.6 months, HR = 0.66; 95% CI = 0.55-0.80; $P < 0.0001$), PFS (12.0 *versus* 8.3 months, HR = 0.76; 95% CI = 0.63-0.91; $P < 0.01$) and ORR (42.1% *versus* 26.3%, respectively; $P = 0.0001$) in intermediate-poor-risk mRCC patients. The exploratory analysis of the efficacy of the combination nivolumab plus ipilimumab compared to sunitinib in favorable-risk patients showed no benefit in OS (HR 1.19, 95% CI 0.77-1.85, $P = 0.44$) and as expected, the median OS was not reached in either group. Duration of response was longer with nivolumab plus ipilimumab (HR, 0.46-0.54), and more patients achieved complete response (10.1%-12.8% *vs* 1.4%-5.6%) regardless of risk group (12). Certain histologic features, as sarcomatoid histology, are associated with worse clinical outcomes. Up to 20% of mRCC patients present a sarcomatoid dedifferentiation. Since this subtype express high level of PD-1 and PD-L1, immunotherapy represents a promising therapy for these patients. A *post-hoc*

analyses of Checkmate 214 was conducted focusing on intermediate-poor risk, advanced clear-cell RCC with sarcomatoid features. The descriptive analyses performed at a minimum follow-up of 30 months, confirmed promising efficacy in terms of ORR (56.7% *versus* 19%) and complete response rate (18.3% *versus* 0), OS (31.2 *versus* 13.6, HR 0.55), and PFS (8.4 *versus* 4.9 months, HR = 0.61) with nivolumab plus ipilimumab compared to sunitinib in previously untreated, intermediate-poor risk, advanced clear-cell RCC with sarcomatoid features (25). In the primary analysis treatment-related adverse events (TRAE) occurred in 93% of patients treated with nivolumab plus ipilimumab and in 97% of patients treated with sunitinib. Grade 3 or 4 events occurred 46% and 63% of patients, respectively. TRAE events leading to discontinuation occurred in 22% and 12% of the patients in the respective groups. No new safety signals emerged to long-term follow-up. Concerning the issue of survival in patients who had to discontinue immunotherapy for TRAE, Tannir *et al.* conducted a *post-hoc* analysis. The analysis showed that a benefit in OS persisted in patients despite having to discontinue therapy due to adverse events (26).

Antiangiogenic and immunotherapy combinations

Immunotherapy was also combined with antiangiogenic treatment due to their synergistic effect. Immunosuppression and angiogenesis represent the key mechanism in mRCC pathogenesis. The two mechanisms interact with each other determining changes in tumor microenvironment (TME). The complex interconnection between TME and immune system was exploited to enhance the immune responses obtained by ICIs alone. The VEGFR signal blockade, in fact, leads to a modulation and recovery of TME and host immunity useful to enhance the anti-tumor immune response. Combining immunotherapy with antiangiogenic treatment in fact, showed to improve outcomes in mRCC patients compared to TKI monotherapy.

The KEYNOTE-426 trial is a large phase III trial that enrolled 861 patients to show a superiority of the combination of pembrolizumab (200 mg every 3 weeks for up to 35 cycles) plus axitinib (5 mg orally twice daily) over sunitinib (50 mg for 4 weeks in 6-week cycles) in untreated mRCC patients. Initial data showed an improvement in OS (90% *versus* 78%, HR = 0.53; 95% CI = 0.38-0.74; $P < 0.0001$) and ORR (59% *versus* 36%, $P < 0.001$) with pembrolizumab plus axitinib across all the IMDC risk groups and regardless of PD-L1 expression (15). The updated data presented at the 2020 annual ASCO congress confirmed the superiority of the combination over sunitinib at the median follow-up of 23 months. Considering the intention-to-treat popu-

lation, median OS was not yet reached for the patients assigned to receive the combination *versus* 35.7 months for the patients assigned to sunitinib (HR = 0.68; 95% CI = 0.55-0.85 $P < 0.001$). Median PFS was 15.4 months and 11.1 months in pembrolizumab plus axitinib arm and sunitinib arm respectively (HR = 0.71; 95% CI = 0.60-0.84, $P < 0.001$). In addition, the ORR was 60.2% with the combination and 40% in the sunitinib arm, with a complete response rate of 9% *versus* 3%. Grouping patients by IMDC risk, significant differences in OS and PFS (HR of 0.63 for OS and 0.69 for PFS) were observed for patients with intermediate-poor risk disease, while no significant differences in OS or PFS were observed for patients with favorable-risk disease. However, the favorable-risk group had a better ORR (69.6 *versus* 50.4% and 55.8 *versus* 35.2%, respectively) and a higher complete response rate (11% *versus* 6% and 8 *versus* 2%, respectively) with pembrolizumab plus axitinib *versus* sunitinib than did the intermediate-poor group (16).

In the JAVELIN phase III trial 442 treatment-naïve mRCC patients were randomly assigned to receive a PD-L1 inhibitor, avelumab (10 mg/kg intravenous infusion every 2 weeks) and a multikinase inhibitor, axitinib (5 mg orally twice daily) or sunitinib (50 mg orally once daily schedule 4 weeks on and 2 weeks off) (13). The two independent primary endpoints were PFS and OS among patients with PD-L1+ tumors (defined as $\geq 1\%$ of immune cells staining positive within the tumor area of the test-

TRIAL CLINICALTRIALS.GOV	PHASE AND DESIGN	TREATMENT ARM	COMPARISON ARM	SETTING	PRIMARY ENDPOINT
CLEAR NCT02811861	III, randomized, open label	Lenvatinib + Everolimus or Pembrolizumab	Sunitinib	First line mRCC	PFS by independent review
COSMIC-313 NCT03937219	III, randomized, open label	Cabozantinib + Nivolumab + Ipilimumab	Nivolumab + Ipilimumab	First line, intermediate- or poor-risk mRCC	PFS per blinded independent central review
PDIGREE STUDY NCT03793166	III, randomized, open label	Cabozantinib + Nivolumab	Nivolumab + Ipilimumab	First line mRCC	OS
NCT03149822	I/II, open label, single arm	Pembrolizumab + Cabozantinib		First or second line mRCC	ORR (CR + PR)
NCT03200587	Ib, open label	Avelumab + Cabozantinib		First line mRCC	DLTs, AEs, RP2D

Table II. Ongoing clinical trials evaluating antiangiogenics and immune checkpoint inhibitors combinations in metastatic renal cell carcinoma. List of terms: AEs, adverse events; CR, complete response; DLT, dose limiting toxicity; mRCC, Metastatic Renal Cell Carcinoma; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PR partial response; RP2D, recommended Phase 2 dose.

ed tissue sample) (13). Key secondary endpoints were OS and PFS in the overall population. At a minimum follow-up of 13 months, among the patients with PD-L1-positive median PFS was 13.8 *versus* 7.0 months (HR = 0.62, 95% CI = 0.490-0.777; $P < 0.0001$) and ORR was 55.9% *versus* 27.2% with avelumab plus axitinib and sunitinib respectively (13). Considering the overall population, PFS was 13.3 *versus* 8.0 months in the avelumab plus axitinib and sunitinib arms, respectively (HR = 0.69, 95% CI = 0.5740.825; one-sided $P < 0.0001$) (13). In conclusion, the combination showed to be superior in terms of PFS irrespective of IMDC risk group and PD-L1 expression. OS data were immature (13). TRAE occurred in 99.5% *versus* 99.3% of patients treated with avelumab plus axitinib *versus* sunitinib respectively (13). Discussing safety data, the events were G3 or higher in 71.2% and 71.5% of the patients in the respective groups (13). The combination nivolumab plus cabozantinib recently demonstrated to be very promising. The CheckMate-9ER tested the combination and results were presented very recently at the ESMO 2020 annual congress. The positive results support the increasing number of data showing that TKIs may create a more immune-permissive tumor microenvironment that could enhance the response to checkpoint. The combination was recently approved by FDA. In detail, CheckMate-9ER is the phase III trial, randomizing 651 previously untreated mRCC patients to receive nivolumab 240 mg intravenous infusion every 2 weeks plus cabozantinib 40 mg orally daily or sunitinib 50 mg daily (schedule 4 weeks on and 2 weeks off). Primary endpoint was PFS. Secondary endpoint were OS, ORR and safety. The study met its primary endpoint showing a consistent benefit in terms of PFS for the combination of nivolumab plus cabozantinib over sunitinib (16.6 *versus* 8.3 months HR = 0.51; 95% CI = 0.41-0.64, $P = 0.0001$). The superiority of nivolumab plus cabozantinib over sunitinib was observed also in terms of ORR (55.7% *versus* 27.1% $p < 0.0001$) with complete response rate of 8.0% *versus* 4.6%. The benefit in PFS and ORR was demonstrated in numerous subgroups including age, sex, PD-L1 expression, bone metastases, and IMDC risk group. The combination was well tolerated and reflected the known safety profiles of both nivolumab and cabozantinib. TRAE for any grade occurred in 97% *versus* 93% of patients treated with nivolumab plus cabozantinib *versus* sunitinib respectively. The events were G3 or higher in 61% *versus* 51%

of patients in the respective groups. Moreover, patients treated with nivolumab plus cabozantinib reported significantly better health-related quality of life than those treated with sunitinib at most time points, according to National Comprehensive Cancer Network-Functional Assessment of Cancer Therapy (NCCN-FACT) Kidney Symptom Index 19 (FKSI-19) scores. Longer-term data for OS are certainly needed because they are still immature (27). All the studies mentioned above combining immunotherapeutic drugs or checkpoint inhibitors with a TKI included clear cells carcinoma and excluded rare histologies such as collecting duct carcinoma or papillary tumors.

DISCUSSION

The approval of therapeutic combos as treatment for mRCC is rapidly changing the clinical practice. The rationale for combining different therapies was rooted in cancer-immunity cycle. The cycle consists into a series of functional stepwise events involving stimulatory and inhibitory factors to obtain an efficient control of cancer growth by the immune system (19). The synergistic effect that can result from the combination of two different therapies has been exploited to enhance the anti-tumor immune response obtained by ICIs monotherapy (28). In detail, the CTLA-4 inhibition, which leads to an active immune response at the level of T-cell proliferation has a synergistic effect with PD-1 inhibition, which modulates the immune response at the level of the tumor bed (19). On the other hand, combining ICIs with VEGF- or VEGFR-directed therapy significantly improved the outcomes of mRCC patients compared to TKI monotherapy. The VEGFR signal blockade, in fact, exert immunomodulatory activities, recovering TME and host immunity. This effect permitted to enhance the anti-tumor immune response obtained by ICIs alone (28). We are moving into a phase where different and effective therapies will be available, without knowing how to select patients. For this purpose, having predictive biomarkers to guide therapeutic decisions will be critical. Direct comparison of studies leading to approval of the combinations should be avoided (11, 13, 15). However, we can derive useful information. Treatment choices could in fact be based on different clinical insights deriving from the characteristics of patients, of disease or drug activity. Concerning drug activities, we know that safety profile

or timing of action is different for TKI and immunotherapy. In detail, ICIs are associated with meaningful long-lasting responses (11). The CheckMate 214 trial confirmed the long-term benefit of the immunotherapy combination nivolumab plus ipilimumab in intermediate-poor risk at 42 months minimum follow-up. The study showed no separation in curves for the first months but a survival plateau occurs at 24-months, meaning that responses with the immune-combo are durable on the long-term compared to sunitinib, where response continues to decline (11). Compared to CheckMate 214, the follow-up with pembrolizumab plus axitinib is not so long yet. However, the OS curves separate since the beginning compared to sunitinib, meaning that the combination starts to work early. Therefore, we can derive that the combination ICIs-TKI could be preferred when shrinkage is needed for aggressive or rapidly progressive disease or for symptomatic patients (15). On the other hand, ipilimumab plus nivolumab or the combo cabozantinib plus nivolumab could be preferred if complete response is the prefixed objective. However, a high percentage of progressive disease as best response were observed in CheckMate 214 (11, 27, 29, 30).

Safety profile could also be helpful in clinical practice. In the CheckMate 214, 25% of patients discontinued treatment due to TRAEs, the most occurring during the induction phase. In the Keynote 426, G3 or higher adverse events of any cause occurred in 66% and 62.4% of patients in the pembrolizumab plus axitinib group and in the sunitinib group, respectively. Moreover, axitinib is given full dose, different from the 9ER trial, where immunotherapy is combined to cabozantinib at reduced dose from the beginning (11, 15, 27). Safety issue for TKI-immuno combo unfortunately, tend to persist over time due to prolonged administration of both agents. The possibility of using a TKI just for a limited period according to a possible intermittent schedule are under investigation with the aim to avoid overtreatment and reduce toxicity and costs. Ultimately, the quest for optimal methods to select patients may involve refinement of tissue-based

analysis, angiogenesis and inflammatory gene expression signatures and blood-based biomarkers, among others (31, 32).

The IMmotion150 trial investigating the combination with the angiogenesis inhibitor bevacizumab and the PD-L1 inhibitor atezolizumab showed different biologic subgroups based on gene expression (33). Effector T cells, interferon γ or angiogenesis gene expression signatures, could be used to select patients more responsive to the immunotherapy combinations over TKIs and *viceversa*. Moreover, favorable risk patients showed a more angiogenic phenotype, suggesting that VEGF agents could remain a good treatment choice (33). Question about the optimal sequencing remains open and prospective trial investigating innovative therapies are needed. Patients rapidly progress on therapy, so improved therapeutic option with superior efficacy are needed and enrolment into clinical trials investigating promising therapies with new mechanism of action is recommended.

CONCLUSIONS

The treatment landscape of mRCC is rapidly evolving, but several unmet needs remain. We are moving into a phase where different therapies are available, without knowing how to select patients. Certainly, the identification of predictive biomarker to guide therapeutic decisions is essential. Going forward, tissue-based analysis, whole genome sequencing and epigenetic analysis will probably help to understand the biology of RCC and to distinguish genomic signatures that can predict response to different treatment strategies.

CONFLICT OF INTERESTS

The authors have declared no conflict of interests.

REFERENCES

1. Capitanio U, Montorsi F. Renal cancer. *Lancet* 2016;387:894–906.
2. Escudier B, Eisen T, Stadler WM, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007;356:125–4.
3. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007;356:115–24.
4. Rini BI, Halabi S, Rosenberg JE, et al. Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. *J Clin Oncol* 2010;28(13):2137–43.
5. Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2010;28:1061–8.
6. Choueiri TK, Escudier B, Powles T, et al. Cabozantinib versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015;373:1814–23.
7. Motzer RJ, Escudier B, Oudard S, et al. Phase 3 trial of everolimus for metastatic renal cell carcinoma: final results and analysis of prognostic factors. *Cancer* 2010;116:4256–65.
8. Choueiri TK, Hessel C, Halabi S, Sanford B, Michaelson MD, Hahn O, et al. Cabozantinib versus sunitinib as initial therapy for metastatic renal cell carcinoma of intermediate or poor risk (Alliance A031203 CABOSUN randomised trial): Progression-free survival by independent review and overall survival update. *Eur J Cancer* 2018;94:115–125. Erratum in: *Eur J Cancer* 2018;103:287.
9. George DJ, Hessel C, Halabi S, et al. Cabozantinib versus sunitinib for untreated patients with advanced renal cell carcinoma of intermediate or poor risk: subgroup analysis of the alliance A031203 CABOSUN trial. *Oncologist* 2019;24(11):1497–1501.
10. Motzer RJ, Tykodi SS, Escudier B, et al. Final analysis of the CheckMate 025 trial comparing nivolumab (NIVO) versus everolimus (EVE) with >5 years of follow-up in patients with advanced renal cell carcinoma (aRCC). *J Clin Oncol* 2020;38(suppl_6):617.
11. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomized, controlled phase 3 trial. *Lancet Oncol* 2019;20(10):1370–85.
12. Tannir NM, McDermott DF, Escudier B, et al. Overall survival and independent review of response in CheckMate 214 with 42-month follow-up: first-line nivolumab + ipilimumab (N+I) versus sunitinib (S) in patients (pts) with advanced renal cell carcinoma (aRCC). *J Clin Oncol* 2020;38(6_suppl):609.
13. Choueiri TK, Motzer RJ, Rini BI, et al. Updated efficacy results from the JAVELIN Renal 101 trial: first-line avelumab plus axitinib versus sunitinib in patients with advanced renal cell carcinoma. *Ann Oncol* 2020;31(8):1030–9.
14. Rini BI, Powles T, Atkins MB, et al. Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. *Lancet* 2019;393(10189):2404–15.
15. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med* 2019;380(12):1116–27.
16. Plimack ER, Rini BI, Stus V, et al. Pembrolizumab plus axitinib versus sunitinib as first-line therapy for advanced renal cell carcinoma (RCC): updated analysis of KEYNOTE-426. *J Clin Oncol* 2020;38(15_suppl):5001.
17. Zhang S, Zhang E, Long J, et al. Immune infiltration in renal cell carcinoma. *Cancer Sci* 2019;110(5):1564–72.
18. Miller JF, Sadelain M. The journey from discoveries in fundamental immunology to cancer immunotherapy. *Cancer Cell* 2015;27(4):439–49.
19. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* 2013;39(1):1–10.
20. Kwon B. Is CD137 ligand (CD137L) signaling a fine tuner of immune responses? *Immune Netw* 2015;15(3):121–4.
21. Seidel JA, Otsuka A, Kabashima K. Anti-PD-1 and anti-CTLA-4 therapies in cancer: mechanisms of action, efficacy, and limitations. *Front Oncol* 2018;8:86.
22. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. [Published correction

- appears in *N Engl J Med* 2010;363(13):1290]. *N Engl J Med* 2010;363(8):711–23.
23. Marinelli O, Annibali D, Aguzzi C, et al. The controversial role of PD-1 and its ligands in gynecological malignancies. *Front Oncol* 2019;9:1073.
 24. Chevrier S, Levine JH, Zanutelli VRT, et al. An immune atlas of clear cell renal cell carcinoma. *Cell* 2017;169:736–49.
 25. McDermott DF, Choueiri TK, Motzer RJ, Arén Frontera O, et al. CheckMate 214 Post Hoc Analyses of Nivolumab Plus Ipilimumab or Sunitinib in IMDC Intermediate/Poor-Risk Patients With Previously Untreated Advanced Renal Cell Carcinoma With Sarcomatoid Features. *J Clin Oncol* 2019;37(15_suppl):4513.
 26. Tannir NM, McDermott DF, Escudier B, Hammers HJ, et al. Overall survival and independent review of response in CheckMate 214 with 42-month follow-up: First-line nivolumab + ipilimumab (N+I) versus sunitinib (S) in patients (pts) with advanced renal cell carcinoma (aRCC). *J Clin Oncol* 2020;38(6_suppl):609.
 27. Choueiri TK, Powles T, Burotto M, et al. Nivolumab + cabozantinib vs sunitinib in first-line treatment for advanced renal cell carcinoma: first results from the randomized phase 3 CheckMate 9ER trial. *Ann Oncol* 2020;31(suppl_4):S1142–S1215.
 28. Mennitto A, Huber V, Ratta R, et al. Angiogenesis and immunity in renal carcinoma: can we turn an unhappy relationship into a happy marriage? *J Clin Med* 2020;9(4):930.
 29. Sepe P, Mennitto A, Corti F, Procopio G. Immunotherapeutic Targets and Therapy for Renal Cell Carcinoma. *Immunotargets Ther* 2020;9:273-88.
 30. Procopio G, Sepe P, Claps M, de Braud F, Verzoni E. Should we use combination therapy for all advanced renal cell carcinoma? *Lancet Oncol* 2019;20(10):1331-2.
 31. Raimondi A, Sepe P, Claps M, Verzoni E, Procopio G. Do biomarkers play a predictive role for response to novel immunotherapeutic agents in metastatic renal cell carcinoma? *Expert Opin Biol Ther* 2019;19(11):1107–10.
 32. Raimondi A, Randon G, Sepe P, et al. The evaluation of response to immunotherapy in metastatic renal cell carcinoma: open challenges in the clinical practice. *Int J Mol Sci* 2019;20(17):4263.
 33. McDermott DF, Huseni MA, Atkins MB, et al. Clinical activity and molecular correlates of response to atezolizumab alone or in combination with bevacizumab versus sunitinib in renal cell carcinoma. [Published correction appears in *Nat Med*. 2018;24(12):1941]. *Nat Med* 2018;24(6):749–57.



Annals of *Research* in *Oncology*

www.annals-research-oncology.com

DEAR COLLEAGUES,

From March 2021 ***Annals of Research in Oncology*** has started its online publication, reaching one goal: expand the oncology horizon and to encourage high-quality international research.

It publishes rigorously peer-reviewed manuscripts, providing broad coverage of all aspects of oncology, across a lot of themed sections such as Cancer Genetics, Cancer Immunology and Immunotherapy, Cancer Pharmacology, Cancer Imaging and Radiotherapy, Nutrition and cancer and so on.

We would like to encourage you all to submit your papers to the Journal

The Journal accepts **Research Articles, Opinion Papers, Reviews** and much more. All papers will be subject to normal peer review by an international forum of independent experts. We strive to provide our authors with quick turnaround and publication time.

Please, visit the the official website **www.annals-research-oncology.com** to consult the full instructions and contact the Editorial Office for all the information you may need to complete your manuscript submission:

editorialoffice@annals-research-oncology.com

This is an open invitation and we would be gratified if you would share this information with your colleagues and friends.

We thank you in advance for your attention and look forward to hosting your paper on *Annals of Research in Oncology*.