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EDRA SpA

Via G. Spadolini, 7
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With this excitement we are ready for the next publications of **2022** and once again we need your precious help, to be able to grow even more ***ARO*** in the name of scientific excellence.

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EDITORIAL

TACKLING CANCER AS WE DID WITH COVID19: A GLOBAL CHALLENGE THAT NEEDS A COOPERATIVE EFFORT

A. Giordano[1,2]

[1] Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, PA, USA
[2] Department of Medical Biotechnologies, University of Siena, Siena, Italy

CORRESPONDING AUTHOR:

Antonio Giordano
Sbarro Institute for Cancer Research and Molecular Medicine
Center for Biotechnology
Temple University
1801 N. Broad Street
Philadelphia, PA 19122, USA
E-mail: giordano@temple.edu
ORCID: 0000-0002-5959-016X

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While the world is still facing the COVID19 pandemic, this year start with some good news for what concerns cancer care. On February 2nd 2022, right before another successful World Cancer day that is increasing awareness on all cancer-related themes, US President Joe Biden relaunched the "Moonshot" initiative against cancer, with the aim of reducing the mortality rate by at least 50% over the next 25 years and improving the lives of patients and their families.

The first Moonshot initiative was promoted in 2016 by Barack Obama, who entrusted the mission to Joe Biden (his Vice at the time) as commander of the fight against cancer.

The initiative was named Moonshot after the speech by John F. Kennedy, in which he said: "*We choose to go to the Moon not because it is easy, but because it is difficult*". Indeed, setting difficult goals helps to bring the best energies into play and choose which battle to fight, which to postpone, which to win. The Moonshot relaunched in February 2022 is calling on the private sector, foundations, academic institutions, healthcare providers, and all citizens to participate (see https://www.whitehouse.gov/cancermoonshot/) sharing ideas, setting priorities and pushing for progress. The programme focuses on a wide range of aspects aiming to create a cancer immunotherapy network; to examine why the implemented strategies are effective for some patients and not for others; identify ways to overcome cancer resistance to treatments; build a nationwide cancer data system for researchers, doctors, and patients; encourage research on childhood cancer; reduce the side effects caused by current cancer treatments; ensure early detection and prevention strategies; use precision medicine and build 3D maps to help researchers understand how cells interact and evolve into cancer; finally, to develop new technologies and treatments for cancer.

"*It's bold, it's ambitious, but it's absolutely doable; just as we have used size to develop cutting-edge vaccines and treatments against COVID19, we will bring a strong sense of urgency to the fight against cancer*".

With these words President Biden paves the way for the application of the criteria of urgency, the analogy of feasibility and interconnection to the battle against cancer.

The pandemic showed us that working together toward the same goal, pooling ideas, sharing information, studies, and solutions in science works. The collaboration between states, scientists, doctors at the forefront in a single whole, represented the winning strategy.

By applying this logic to cancer, we will be able to lay the foundations, in the near future, of a reality in which sharing could make a difference. Cancer is also an "emergency", so it should be treated in the same way as COVID19.

The goal is to cure cancer, make it manageable, lengthen and improve the lives of patients, act for prevention, make early diagnoses.

By analogically applying the thrust received in the pandemic, the urgency and the interconnections between minds and studies, establishing the search for a common strategy as a global priority, it will also be possible to identify a possible solution for the "cancer emergency".

At present, the funding scheme of the renewed Moonshot programme is not clear yet, but it is expected to be disclosed soon1, 2.

While we expect much progress stemming from this initiative, we all, as scientists and doctors, as patients or relatives of cancer patients, as citizens as well, should make an effort to promote anticancer strategies starting from our own selves. For example, we are still lagging behind in applying prevention strategies that have been officially formalized long ago (see for example the European code against cancer: https://cancer-code-europe.iarc.fr/index.php/en/). We all should promote healthier lifestyle habits and policies to safeguard the environment. The war against cancer should be everyone everyday battle.

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REVIEW

THE EMERGING ROLE OF IMMUNOTHERAPY IN GASTROESOPHAGEAL CANCER: STATE OF ART AND FUTURE PERSPECTIVE

A. Raimondi[1], M. Prisciandaro[1,2], F. Pagani[1], G. Randon[1], F. Corti[1], F. Nichetti[1], M. Niger[1], F. Morano[1], F. Pietrantonio[1], M. Di Bartolomeo[1]

[1] Department of Medical Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
[2] Department of Oncology and Hematology, University of Milan, Milan, Italy

CORRESPONDING AUTHOR:

Maria Di Bartolomeo
Department of Medical Oncology
Fondazione IRCCS Istituto Nazionale dei Tumori di Milano
via Venezian 1
20133 Milan, Italy
E-mail: maria.dibartolomeo@istitutotumori.mi.it
ORCID: 0000-0002-7954-6609

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ABSTRACT

The introduction of immunotherapy in the therapeutic algorithm of gastroesophageal cancer is still a debated issue. Recent findings from randomized clinical trials documented the efficacy of adjuvant nivolumab in improving disease free survival (DFS) in resectable esophageal and gastroesophageal junction cancer patients with residual pathologic disease after neoadjuvant chemoradiation (CheckMate 577). Consistently, the combination of pembrolizumab and doublet chemotherapy with 5-fluorouracil plus cisplatin improved first-line treatment outcomes in metastatic esophageal squamous cancer; moreover the major benefit was observed in tumor expressing PD-L1 combined positive score (CPS) > 10 (Keynote 590). Finally, the addition of nivolumab to first-line oxaliplatin and 5-fluorouracil-based chemotherapy improved overall survival, progression free survival and response rate in patients with metastatic gastric/gastroesophageal junction cancer with PD-L1 positive score (PD-L1 CPS ≥ 5) (CheckMate 649). Moving forward, the research focused on the identification of predictive biomarkers of response to immunotherapy, to refine the patients' selection and maximize the treatment benefit. Microsatellite instability has been shown to predict higher response to checkpoint inhibitors as highlighted by subgroup analyses of the pivotal studies. For what concerns microsatellite stable tumors, the expression of PD-L1, the positivity for Epstein-Barr virus and a high tumor mutational burden are now regarded as the most promising and reliable predictive markers for immunotherapy as far as now. Therefore, the anti-PD1 agents nivolumab and pembrolizumab proved to confer

an improvement in the outcome of gastroesophageal cancer patients but the real magnitude of benefit of immunotherapy in this disease setting is under definition. Biomarker-focused research will allow clinicians to define the optimal therapeutic algorithm in the different patients populations.

KEY WORDS

Immunotherapy; biomarker; gastric cancer; PD-L1; microsatellite instability.

IMPACT STATEMENT

Immune-checkpoint inhibitors proved to confer a meaningful benefit in the setting of gastric/gastroesophageal junction cancer, nevertheless a refinement of patients selection according to predictive biomarkers could maximize the treatment benefit.

INTRODUCTION

Gastroesophageal cancer (GC) is a highly aggressive tumor that ranks at the sixth place for the incidence of new cancers worldwide (about 1,033,000 cases) and represents the third most common cause of cancer-related death, resulting in approximately 780,000 deaths yearly (1). The epidemiology of GC widely varies according to the geographical region, and, specifically, in Europe the incidence is estimated at 81,600 and 51,500 cases in men and women, respectively, and the number of deaths is rated at 62,000 in men and 40,300 in women (2). In Italy 14,500 new diagnoses were estimated in 2020 and about 8,500 deaths were estimated in 2021, respectively (3).

The cornerstone of potentially curative treatment in non-metastatic disease is radical surgery, combined with peri-operative or adjuvant chemotherapy according to International Guidelines (4-10). Nowadays, both the adjuvant and peri-operative chemotherapy schedules are evidence-based and guideline-endorsed treatments, although in Asia the preferred approach is surgery plus adjuvant chemotherapy, whereas outside of Asia peri-operative chemotherapy is the most frequent choice (11). Despite the improvements in the disease management thanks to the development of multimodality treatment strategies, more than half patients still relapse and die from their disease. Nowadays, GC/gastroesophageal junction cancer (GEJC) remains one of the most lethal malignancies with 5-year survival rates of about 22% and less than 4% for localized and metastatic disease, respectively (2). In the setting of metastatic GC/GEJC the choice of the optimal first-line chemotherapy is based upon, on the one side, the extension and molecular characteristics of tumor, mainly the presence of HER2 overexpression/amplification, and on the other side, the clinical conditions and comorbidities of patients (11). The doublet combination with platinum derivative and fluoropyrimidine is considered a standard of care with or without trastuzumab (in case of HER2 overexpressed/amplified tumors). In the second-line setting and in later treatment lines, the combination of taxanes plus ramucirumab, ramucirumab monotherapy or irinotecan represent the main choices, even though with poor survival outcomes (12-17). The research progresses led to a deeper understanding of the molecular characterization of GC, providing the opportunity to classify tumors into different subtypes on the basis of their genomic profile, with the most common TCGA classification, furtherly described in the **table I** (18).

The immunotherapy revolution deeply changed the therapeutic management and the prognosis of patients in several cancer settings, such as non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma (RCC), urothelial cancer and head and neck tumors (19). The principal therapeutic weapon is represented by immune checkpoint inhibitors (ICIs) targeting the programmed cell death receptor 1 and its ligand (PD1 and PD-L1). In fact, tumors upregulate the inhibitory checkpoints of the immune system, while ICIs release the brakes and reactivate T-cells activity in order to promote the anti-tumor immune reaction(20). In the setting of GC/GEJC, several studies have been conducted or are ongoing to explore and define the potential role of ICIs. In this review we aim at depicting a comprehensive picture of the current scenario and of the future perspectives.

LOCALIZED DISEASE

In the setting of localized or locally advanced disease, eligible for curative radical surgery, few data have been collected on the role of immunotherapy. First of all, in stage II/III esophageal or GEJC patients treated with chemoradiotherapy followed by surgery and with evidence of residual disease, adjuvant treatment with nivolumab for 1 year provided a statistically significant and clinically meaningful advantage in disease-free survival (DFS) (median DFS 22.4 *vs* 11.0 months, HR 0.69, 95% CI 0.56-0.86, p ≤ 0.001) over placebo in the phase III CheckMate 577 trial. Disease-free survival favored nivolumab across multiple prespecified subgroups, and the benefit was more pronounced in the squamous histotype (median DFS 29.7 *vs* 11.0 months, HR 0.61, 95% CI 0.42-0.88) although maintained also in the adenocarcinoma subtype (median DFS 19.4 *vs* 11.1 months, HR 0.75, 95% CI 0.59-0.96), potentially opening a new therapeutic scenario (21).

Moving forward, clinical trials are ongoing in order to provide evidence-based results. In details, the randomized, open-label, phase II DANTE study (NCT03421288) is investigating the combination of the anti-PD-L1 agent atezolizumab to peri-operative FLOT regimen (5fluorouracil, oxaliplatin and docetaxel), followed by adjuvant atezolizumab, versus standard peri-operative FLOT in GC or GEJC (Siewert I-III) cT2 or higher, any N or node positive, without any biological selection and HER2 status not assessed. The randomization is stratified per microsatellite instability (MSI) status while PD-L1 expression is performed but does not represent a stratification factor. The study completed the recruitment and the presented safety results showed that the chemo-immuno regimen was safe and feasible in the peri-operative setting of GC/GEJC, while activity and efficacy results are not available yet (22). Similarly, the randomized, double-blind, phase III KEYNOTE-585 study (NCT03221426) is investigating pembrolizumab or placebo combined with peri-operative chemotherapy, followed by pembrolizumab or placebo maintenance in T3 or higher or N positive GC/GEJC patients. The initial chemotherapy schedule was cisplatin plus 5fluorouracil or capecitabine, but the study was amended to include a cohort with FLOT after the results of the FLOT4 trial (9). The trial will assess the status of MSI and PD-L1 as exploratory biomarkers, though neither MSI status nor PD-L1 represent stratification factors. The two above-described trials are investigating immunotherapy in an unselected population. However, the results of the recent pivotal trials conducted in the metastatic setting highlighted how predictive biomarkers of response to immunotherapy are crucial to select patients with predicted enhanced response to ICIs. Particularly, as discussed above, agnostic tumors with MSI-high status are highly responsive to immunotherapy, thus clinical trials are ongoing in this peculiarly selected subpopulation (23, 24). The rationale relies in the results of proof of concept studies that showed how pre-operative immunotherapy could achieve a pathologic major or complete response in potentially resectable mismatch repair deficient (dMMR)/MSI-high tumors and eventually provide a chance of cure even regardless

SUBTYPE	FREQUENCY	CHARACTERISTICS
Chromosomal Instability (CIN)	50%	• Intestinal histology • TP53 mutation • High frequency of tyrosine kinase/RAS pathway activation
Genomically Stable (GS)	20%	• Diffuse histology • CDH1, RHOA mutations • CLDN18-ARHGAP fusion • alterations in cellular adhesion molecules genes
Epstein-Barr Virus (EBV)	9%	• PIK3CA mutation • PD-L1/2 overexpression • EBV-CIMP • CDKN2A silencing • Immune cell signalling
Microsatellite Instability (MSI),	22%	• Hypermutation • Gastric-CIMP • MLH1 silencing • Mitotic pathways

Table I. *Description of The Cancer Genome Atlas Classification (TCGA) of gastric cancer.*

of surgery. In details, in the phase II NICHE study, a window of opportunity treatment with 1 cycle of ipilimumab plus nivolumab in resectable colorectal cancer patients obtained no meaningful response in pMMR cases while a major or complete pathological response in all but one dMMR ones (25). This was confirmed by a case series of localized MSI-high GC or colon cancer patients achieving a high rate of pCR after immunotherapy (26). On this basis, two trials are ongoing to test immunotherapy in MSI-high GC/GEJC patients eligible for radical surgery. The first one is the GERCOR NEONIPIGA trial (NCT04006262) that is aimed at enrolling 32 patients to receive a 12-week preoperative combo-immunotherapy with nivolumab plus ipilimumab and, after radical surgery, postoperative nivolumab up to 1 year. The second one is the Italian, multicenter, single-arm, multicohort, phase II INFINITY study (NCT04817826) aimed at investigating the safety and activity of the ICIs combination durvalumab (1500 mg q4w for 3 cycles) plus tremelimumab (300 mg single dose) as preoperative or potentially definitive treatment in dMMR/MSI-high/Epstein-Barr (EBV) negative GC/GEJC patients. The Cohort 1 is enrolling up to 18 patients and its primary endpoint is the rate of pCR at surgery after neoadjuvant immunotherapy, while Cohort 2 will investigate a non-operative-management strategy in patients achieving complete clinical response at radiological, tissue and liquid biopsy level after immunotherapy (**figure 1**) (27).

UNTREATED METASTATIC DISEASE

In the setting of first-line treatment for advanced/metastatic GC/GEJC, the first study was the non-randomized, multicohort, phase II KEYNOTE-059 study that investigated pembrolizumab in combination with standard cisplatin-fluoropyrimidine chemotherapy irrespectively of PD-L1 expression in Cohort 2 and pembrolizumab monotherapy in patients with PD-L1 combined positive score (CPS) ≥ 1 in Cohort 3. Overall, 25 and 31 patients were enrolled in Cohort 2 and 3, respectively: the ORR was 60.0% (95% CI, 38.7-78.9) and 25.8% (95% CI 11.9-44.6), median duration of response was 4.6 and 9.6 months, and median overall survival (OS) was 13.8 and 20.6 months, respectively, with a globally manageable tolerability profile (28).

On this basis, the randomized, phase III KEYNOTE-062 trial was designed and conducted in treatment naïve advanced GC/GEJC Asian and non Asian patients selected for PD-L1 expression CPS ≥ 1. A total of 763 patients were randomized 1:1:1 to pembrolizumab monotherapy versus pembrolizumab plus standard cisplatin-fluoropyrimidine chemotherapy versus placebo plus chemotherapy. The complex statistical design of the study compared pembrolizumab to placebo plus chemotherapy, showing the non-inferiority (primary endpoint) (median OS 10.6 *vs* 11.1 months, HR 0.91, 99.2% CI 0.69-1.18) but not the superiority of pembrolizumab as compared to chemotherapy. Nevertheless, it should be pointed out that at least half of fit-for-a-trial patients treated with pembrolizumab died earlier than with chemotherapy and that the accepted confidence interval for inferiority margin worse than chemotherapy was wide, besides the absence of an improvement in quality of life. Moreover, the addition of pembrolizumab to chemotherapy failed to condition an improvement

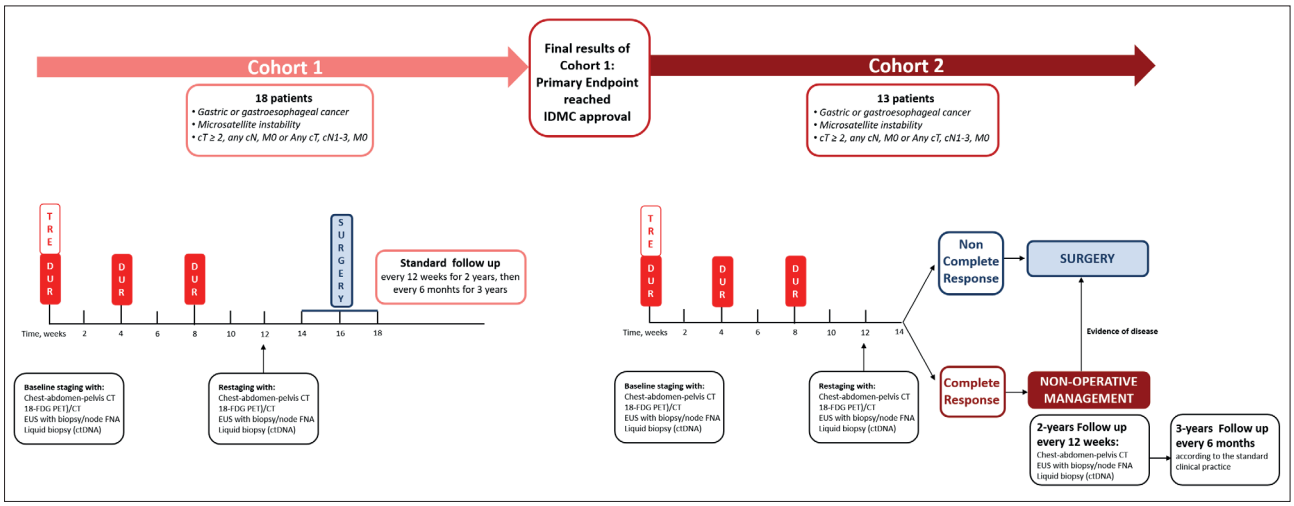

Figure 1. *Study Diagram.*

in terms of OS over standard chemotherapy alone both in patients with CPS ≥ 1 (median OS 12.5 *vs* 11.1 months, HR 0.85, 95% CI 0.70-1.03, p = 0.05) and CPS ≥ 10 (median OS 12.3 *vs* 10.8 months, HR 0.85, 95% CI 0.62-1.17, p = 0.16) (29).

Afterwards, the randomized, open-label, phase III JAVELIN Gastric 100 study enrolled Asian and non Asian advanced or metastatic GC/GEJC patients (independently from PD-L1 expression) who achieved disease control after a 12-week first-line therapy with platinum/fluoropyrimidine, and compared the switch maintenance with avelumab versus the continuation of the standard treatment. Avelumab failed to achieve the superiority in terms of OS (median OS 10.4 *vs* 10.9 months, HR 0.91, 95% CI 0.74-1.11, p = 0.1779) in the overall trial population and in the PD-L1 positive on tumor cells subgroup. The possible caveats could be found in the duration of "induction" first-line chemotherapy (12 weeks only) and in the selection of patients (with TPS instead of CPS), since in an exploratory analysis stratifying patients for PD-L1 CPS ≥ 1 and < 1, a survival advantage was reported (median OS 14.9 *vs* 11.6 months, HR 0.72, 95% CI 0.49-1.05), even though not confirmed with the cutoff of CPS ≥ 10 (30).

Recently, three pivotal studies have been presented, with potentially practice-changing results. First, the randomized, open-label, phase III CheckMate 649 trial enrolled previously untreated, advanced or metastatic, HER2 negative, Asian and non Asian GC/GEJC patients, regardless of PD-L1 expression, who were randomized to ipilimumab plus nivolumab, nivolumab plus XELOX/FOLFOX or XELOX/FOLFOX. Afterwards, the combo-immuno arm was closed and the primary population was amended to cases with PD-L1 CPS ≥ 5. The combination of nivolumab to standard first-line chemotherapy succeeded in significantly improving OS (median OS 14.4 *vs* 11.1 months, HR 0.71, 95% CI 0.59-0.86, p < 0.0001) and progression-free survival (PFS) (7.7 *vs* 6.0 months, HR 0.68, 95% CI 0.56-0.81, p < 0.0001) over chemotherapy alone in patients with CPS ≥ 5. Consistently, the OS outcome was significantly improved with the addition of nivolumab to first line in patients with CPS ≥ 1 and in the overall study population, with a manageable safety profile, thus the Food and Drug Administration (FDA) approved this schedule independently from the expression of PD-L1 (31). Nevertheless, it should be remarked that the magnitude of benefit, in terms of delta of OS improvement and HR, progressively decreased from CPS ≥ 5 to CPS ≥ 1 to overall, and, notably, there was an enrichment of patients with CPS ≥ 5 in the two latter populations (about 70% in CPS ≥ 1 and 60% in all patients randomized). Therefore, the results in patients with CPS < 1 or between 1 and 5 would possibly provide interesting insights on the real benefit of immunotherapy in the different subgroups of patients. On the other hand, the immunotherapy combination ipilimumab plus nivolumab failed to significantly improve OS over chemotherapy in the CPS ≥ 5 subgroup and the curves showed the typical crossing, suggesting that a chemotherapy-free regimen should not be the choice for the upfront treatment in metastatic GC/GEJC (32).

Second, the randomized, placebo-controlled, phase II/III ATTRACTION-4 study randomized only Asian advanced/metastatic GC/GEJC patients to nivolumab or placebo plus oxaliplatin and capecitabine or S1 irrespectively of the expression of PD-L1. While the phase II part of the study showed promising results for the chemo-immunotherapy combination, the phase III part reported a statistically significant improvement in terms of PFS (median PFS 10.4 *vs* 8.3, HR 0.68, 95% CI 0.51-0.90, p = 0.0007) while no significant benefit in OS (median OS 17.4 *vs* 17.1, HR 0.90, 95% CI 0.75-1.08, p = 0.257) (33, 34). It could be argued that the different results obtained in CheckMate 649 and ATTRACTION-4 studies, very similar for design and treatment schedule, may be partially explained by the different selection of patients (according or independently to PD-L1 CPS) and by the variable weight of further treatment lines, especially with immunotherapy, higher in the Asian population.

Finally, the randomized, placebo-controlled, phase III Keynote-590 trial compared pembrolizumab plus cisplatin/5-fluorouracil versus placebo plus cisplatin/5-fluorouracil chemotherapy in patients with previously untreated advanced unresectable or metastatic esophageal or gastroesophageal junction carcinoma either adenocarcinoma or squamous cell carcinoma. The study demonstrated that the combination of immunotherapy to the standard first-line chemotherapy provides a statistically significant benefit in terms of OS irrespectively of CPS status, although the magnitude of benefit was higher in patients selected for CPS ≥ 10 or squamous histology and the highest in squamous cell carcinoma with CPS ≥ 10. These results are clinically relevant and conditioned the approval of European Medical Association for patients with untreated advanced esophageal carcinoma with CPS ≥ 10 independently on histology (35). The main results of the pivotal studies are reported in **table II**.

	ATTRACTION-2 N = 330 *VS.* 163	JAVELIN GASTRIC 300 N = 185 *VS.* 186	KEYNOTE-061 N = 296 *VS.* 296	JAVELIN GASTRIC 100 N = 249 *VS.* 250	KEYNOTE-062 N = 256 *VS.* 250 N = 257 *VS.* 250	CHECKMATE 649 N = 789 *VS.* 792	ATTRACTION-4 N = 362 *VS.* 362	KEYNOTE-590 N = 373 *VS* 376
Setting	≥ 3L Unselected GC/GEJC	3L Unselected GC/GEJC	2L CPS ≥ 1 (n = 395) GC/GEJC	1L Maintenance GC/GEJC	1L CPS ≥ 1 GC/GEJC	1L All pts/CPS ≥ 5 GC/GEJC	1L Unselected GC/GEJC	1L Unselected Esophageal/GEJC
Treatment arms	Nivolumab *vs.* Placebo	Avelumab *vs.* CT(inv's choice)	Pembrolizumab *vs.* Paclitaxel	Avelumab *vs.* CT	A: Pembrolizumab *vs.* CT B: Pembrolizumab+CT vs. CT+Placebo	Nivolumab+CT *vs.* CT	Nivolumab+CT *vs.* CT	Pembrolizumab +CT*vs.* CT+Placebo
Response rate	11% *vs.* 0%	2.2% *vs.* 4.3%	15.8% *vs.* 13.6%	13.3% *vs.* 14.4	14.8% *vs.* 37.2% 48.6% *vs.* 37.2%	60% *vs.* 45%	57.5% *vs.* 47.8%	ITT: 45% *vs.* 29.3%
Median PFS, mos	1.61 *vs.* 1.45	1.4 *vs.* 2.7	1.5 *vs.* 4.1	3.2 *vs.* 4.4	2.0 *vs.* 6.4 6.9 *vs.* 6.4	7.7 *vs.* 6.9 7.7 *vs.* 6.0	10.5 *vs.* 8.3	CPS ≥ 10:7.5 *vs.* 5.5 SCC: 6.3 *vs.* 5.8 ITT: 6.3 *vs.* 5.8
Median OS, mos	5.26 *vs.* 4.14	4.6 *vs.* 5.0	9.1 *vs.* 8.3	10.4 *vs.* 10.9	10.5 *vs.* 11.1 12.5 *vs.* 11.1	13.8 *vs.* 11.6 14.4 *vs.* 11.1	17.5 *vs.* 17.2	CPS ≥ 10:13.5 *vs.* 9.4 SCC: 12.6 *vs.* 9.8 ITT: 12.4 *vs.* 9.8
HR (95%CI)	0.63 (0.51-0.78)	1.1 (0.9-1.4)	0.82 (0.66-1.03)	0.91 (0.74-1.11)	0.91 (0.69-1.18) 0.85 (0.70-1.03)	0.80 (0.68-0.94) 0.71 (0.59-0.86)	0.90 (0.75-1.08).	CPS ≥ 10:0.62 (0.49-0.78) SCC:0.72 (0.60-0.88) ITT:0.73 (0.62-0.86)

Table II. *Summary of the main results of the pivotal clinical trials conducted on immunotherapy in the setting of gastroesophageal cancer.*

GC: gastric cancer; GEJC: gastroesophageal junction cancer; CPS: combined positive score; CT: chemotherapy; ITT: intention-to-treat population; SCC: squamous cell carcinoma; PFS: progression-free survival; OS: overall survival; HR: hazard ratio; CI: confidence interval.

PRETREATED METASTATIC DISEASE

In the setting of metastatic GC/GEJC patients refractory to previous treatments, landmark clinical trials have been conducted on the role of immunotherapy, providing conflicting results. First, the randomized, double-blind, phase III ATTRACTION-2 trial investigated the anti-PD1 agent nivolumab (at the dose of 3 mg/kg q14) versus best supportive care in patients with advanced GC/GEJC pretreated with 2 or more lines of therapy, irrespectively of the expression of PD-L1. The study showed a statistically significant improvement in OS for immunotherapy (median OS 5.3 *vs* 4.1 months, HR 0.63, 95% CI 0.51-0.78, p < 0.0001), that displayed an overall response rate (ORR) of 11.4%, with a manageable safety profile. Comparable results were reported in PD-L1 tumor cell (TPS) < and ≥ 1%, that represented about 86.5% and 13.5% of the overall population, with a median OS of 6.0 *vs* 4.2 and 5.2 *vs* 3.8 months, with nivolumab versus best supportive care, respectively. The trial enrolled an only Asian population, therefore nivolumab was approved in third-line setting of GC/GEJC in Asia, while no data are available for non Asian patients, and this is crucial taking into consideration the different tumor biology and the variable sensitivity to immunotherapy in the two populations (36).

Second, the randomized, open-label, phase III JAVELIN Gastric 300 study compared the anti-PD-L1 agent avelumab (at the dose of 10 mg/kg q14) with physician's choice chemotherapy (paclitaxel or irinotecan or best supportive care in patients unfit for chemotherapy) as third-line therapy in advanced GC/GEJC patients both Asian and non Asian. The trial failed to demonstrate a significant benefit in terms of OS with immunotherapy versus standard of care treatment (median OS 4.6 *vs* 5.0 months, HR 1.1, 95% CI 0.9-1.4, p = 0.81), even though with a more favorable safety profile. Negative results were obtained even for the secondary endpoints of PFS and ORR and no differences to remark were found in the subgroup analyses (37).

Third, in the single-arm, multi cohort, open-label, phase II KEYNOTE-059 study, 259 Asian or non Asian patients with GC/GEJC pretreated with 2 or more previous lines were enrolled in Cohort 1 and received the anti-PD1 agent pembrolizumab (200 mg flat dose q21). In this setting, the ICI monotherapy conferred a 11.0% ORR overall and 15.5% *vs* 6.4% in patients with PD-L1 CPS ≥ 1 and < 1, respectively. Therefore, the benefit of immunotherapy was higher in PD-L1 positive patients with durable responses (median duration of response 16.3 months) and median OS of 5.8 months *vs* 4.6 months in PD-L1 negative ones (38). On this basis, pembrolizumab received the approval of FDA for previously treated PD-L1 positive GC patients.

Finally, the randomized, open-label phase III KEYNOTE-061 trial randomized Asian or non Asian GC/GEJC patients having progressed to the first-line treatment to pembrolizumab versus paclitaxel independently from the expression of PD-L1, even though the study was furtherly amended to include only patients with PD-L1 CPS ≥ 1. In patients with CPS ≥ 1, representing about 2/3 of the overall population, pembrolizumab failed to reach a statistically significant improvement in terms of OS over the standard second-line chemotherapy that lacked the combination with the biologic agent ramucirumab (median OS 9.1 *vs* 8.3 months, HR 0.82, 95% CI 0.66-1.03, p = 0.0421) (39). Looking at the curves, about half patients treated with pembrolizumab died before than what occurred with chemotherapy, since curves crossed at 8 months, and the apparent benefit for immunotherapy shown by the tails of the curves may be jeopardized by the limited numbers of patients. Nevertheless, the post-hoc analysis about the stratification for PD-L1 expression provided meaningful results, since in the subgroup analyses for PD-L1, in patients with negative PD-L1 (CPS < 1) pembrolizumab provided worse results than paclitaxel, while in patients with CPS ≥ 10 pembrolizumab was superior to chemotherapy (median OS 10.4 *vs* 8.0 months, HR 0.64, 95% CI 0.41-1.02) (39).

The main results of the pivotal studies are reported in **table II**.

BIOMARKERS

In light of the results of the studies conducted on ICIs in several tumor settings, the research focused on the identification of specific and reliable predictive biomarkers of response to immunotherapy, with the aim of refining the patients' selection and maximizing the treatment benefit. The highest burden of evidence collected on this topic concerns the expression of PD-L1, the status of MSI, the positivity for EBV, the Tumor Mutational Burden (TMB), but the research is going further and new biomarkers are under investigation.

MICROSATELLITE INSTABILITY

The status of MSI-high is a well-established good prognostic factor for prolonged survival in early-stage colorectal cancer patients, and a potential predictive marker of lack of benefit from adjuvant fluoropyrimidine monotherapy in stage II disease (40). In the setting of resectable GC, an Individual Patient Data pooled analysis combining the results of four large international randomized trials (MAGIC, CLASSIC, ARTIST and ITACA-S) was performed and confirmed the powerful positive prognostic effect of MSI-high status in surgically resected GC patients and the predicted lack of benefit of peri-operative or adjuvant chemotherapy after surgery in this molecular subgroup (41). Recently, the key role of MSI status has been established as a powerful predictive marker for responsiveness to immunotherapy since advanced tumors with MSI-high or dMMR status, across different primary sites of origin, proved to be highly responsive to immunotherapy, even more of the other well-known immune-sensitive cancers (23, 24). In fact, the FDA granted an accelerated approval to pembrolizumab for adult and pediatric patients with agnostic unresectable/metastatic MSI-high or dMMR cancers. The explanation lies in the high mutational load of MSI-high tumors, with elevated amount of neoantigens eliciting and boosting the anti-tumor immune response (18, 42). In the specific setting of GC/GEJC, the exploratory analyses of the pivotal clinical trials KEYNOTE-059 and -061 and -062 showed that patients with MSI-high GC had a dramatic benefit in terms of response and survival outcomes from immunotherapy. In details, in Cohort 1 of KEYNOTE-059, the ORR with pembrolizumab monotherapy was 11.6% overall, while 57.1% in MSI-high patients vs 9.0% in MSS ones (38). In KEYNOTE-061 study, patients with MSI-high tumors, irrespectively of PD-L1 CPS, had a median OS not reached (95% CI 5.6 months-not reached) with pembrolizumab *vs* 8.1 months (2.0-16.7) with paclitaxel, and 7/15 patients (47%) achieved an objective response with pembrolizumab *vs* 2/12 (17%) with paclitaxel (39). Finally, in KEYNOTE-062 trial, in the MSI-high subgroup median OS was not reached (95% CI, 10.7-not reached) *vs* 8.5 months (95% CI, 5.3-20.8), median PFS was 11.2 months (95% CI, 1.5-NR) *vs* 6.6 months (95% CI, 4.4-8.3), and ORR was 57.1% *vs* 36.8% with pembrolizumab versus standard chemotherapy, respectively (29). This was confirmed in a meta-analysis including 9 clinical trials and more than 2000 patients, in which MSI-high GC/GEJC treated with anti-PD1 ICIs achieved a higher ORR and disease control rate than MSS ones (43) and even in another meta-analysis of the pivotal first and subsequent treatment lines clinical trials described above (44). Therefore, the significant benefit of immunotherapy in terms of survival outcome in GC/GEJC patients selected for MSI-high status was shown both in first and subsequent treatment lines, as confirmed by the subgroup analysis of the CheckMate 649 study, even though only a minor part of advanced GC/GEJC are MSI-high (about 4%).

EPSTEIN-BARR VIRUS

The recent studies highlighted how the positivity for the EBV in the setting of GC/GEJC represents a powerful biomarker of response to immunotherapy with ICIs, although present in a very limited proportion of advanced GC/GEJC patients, less than 5% (42, 45). For this reason, the available clinical data derive from case reports or series and this biomarker has never been tested in randomized clinical trials. EBV positive is one of the TCGA subtypes, as identified on molecular profiling analyses, characterized by extensive DNA hypermethylation, mutations of PIK3CA and amplifications of CD274 and PDCD1LG2 genes, encoding for PD-L1 and PD-L2, respectively, as well as activation of immune signaling pathways (18). Although EBV-positive tumors are endowed with a low tumor mutational burden, they are characterized by a high expression of immune checkpoints such as PD1 and CTLA-4 and by an elevated histological lymphocytic infiltration (46). Consistently with MSI-high GC/GEJC, EBV-positive ones are endowed with better outcomes after radical surgery than the other subtypes, likely related to the host immune response, and even improved prognosis in the metastatic setting (47). Therefore, results have been obtained on the enhanced sensitivity of EBV-positive tumors to immunotherapy with anti-PD1 and anti-PD-L1 agents, with impressive responses with pembrolizumab in previously treated GC patients (48). Nevertheless, the evidence collected is limited by the low prevalence of this condition, that impairs the opportunity to design and conduct dedicated clinical trials (42, 46).

PD-L1

The first and most investigated biomarker for response to anti-PD1/PD-L1 agents is the expression

of PD-L1, based on the mechanism of activity of ICIs. The clinical significance of the expression of PD-L1 on tumor cells and/or on the immune cells infiltrating the tumor assessed by immunohistochemistry (IHC) was identified in the initial clinical trial investigating the anti-PD1 agent nivolumab and, since then, it has been widely studied in several tumor settings with variable results (19). The rate of PD-L1 expression is highly variable across histologies and the different studies, namely in tumors with enhanced response to immunotherapy, such as NSCLC, melanoma and RCC, it ranges between 14% and 100% and, conversely, in cancers with reduced sensitivity to ICIs, like colorectal cancer or sarcoma, a comparable expression is shown, underlining the potential limitations of this biomarker (49, 50). Furthermore, other crucial limitations of PD-L1 expression may be found in the variability in the methods of assessment and in the tumor heterogeneity. In fact, each anti PD1/PD-L1 ICI has its own companion antibody (*e.g.*, Dako, Leica platform, Ventana Medical System), the scoring systems are not homogeneous for the target cells assessed, whether only tumor cells (Tumor Proportion Score - TPS) or both tumor cells and immune cells infiltrating the neoplastic stroma (Combined Positive Score - CPS), and the definition of the cutoff of positivity is uncertain (51). Additionally, the intra- and inter-tumor heterogeneity should be considered, with potential differential expression between primary tumor and metastases, as well as the possible dynamics of increase and decrease of the expression during the natural history of cancer (52, 53).

In the specific setting of GC/GEJC, the score of reference is the CPS, since it was validated by a comparison with the TPS in the frame of the Cohort 1 of the KEYNOTE-059 study (54). While PD-L1 positive tumors according to TPS ≥ 1% accounted for 12.5% overall with minimal enrichment of responses, CPS ≥ 1 ones represented 57.6% of the total, with meaningful enrichment of responses, besides reaching a high rate of inter and intra-pathologist agreement for the definition of CPS (54). Therefore, the CPS score is currently used in the definition of the study populations, study endpoints and stratification factors of the pivotal clinical trials, as increased PD-L1 expression corresponds to an enhanced tumor response to immunotherapy, even though this does not apply to all cases, with some PD-L1 patients benefitting from ICIs and PD-L1 positive ones not (48). Moreover, the optimal positivity cutoff to discriminate the responsiveness to immunotherapy has not been defined yet (*e.g.* 1, 5, 10). In fact, as discussed above, in the KEYNOTE-061 second-line study, no significant benefit in terms of OS was shown in patients with CPS ≥ 1 (median OS 9.1 *vs* 8.3 months, HR 0.81, 95% CI 0.66-1.00) while an increased benefit was seen with CPS ≥ 5 (10.4 *vs* 8.3 months, HR 0.72, 95% CI 0.53-0.99) and higher with CPS ≥ 10 (10.4 *vs* 8.0 months, HR 0.64, 95% CI 0.41-1.02) (39). Consistently, in the KEYNOTE-062 trial, pembrolizumab was superior in OS to standard chemotherapy in first-line in patients with CPS ≥ 10 (median OS 17.4 *vs* 10.8 months, HR 0.69, 95% CI 0.49-0.97) even though this endpoint could not be formally analyzed due to the statistical design of the study, while the results were negative even for the CPS ≥ 10 subgroup in the pembrolizumab plus chemotherapy versus chemotherapy arm (29). In a recent comprehensive analysis of the pembrolizumab-based trials (KEYNOTE-059, -061 and -062), a consistent improvement was observed in terms of the clinical and survival outcome with pembrolizumab across the different lines of treatment in patients with CPS ≥ 10 (55). Conversely, the cutoff CPS ≥ 5 was chosen for the nivolumab-based CheckMate 649 study, where, as speculated above, the magnitude of benefit of the addition of nivolumab to first-line chemotherapy progressively decreased from the subgroup of CPS ≥ 5, to CPS ≥ 1 to the overall population, possibly suggesting that in the CPS ≥ 1 and whole population the real benefit could have been conditioned by those with CPS ≥ 5, even though the results in patients with CPS < 1 or between 1 and 5 are not available to support this hypothesis (31).

TUMOR MUTATIONAL BURDEN

The tumor mutational burden (TMB) is a recently-defined potential biomarker of response to immunotherapy with ICIs. TMB is defined as the total number of non-synonymous mutations per coding area of tumor genome, as measured as mutations per megabase (mut/Mb). The genomic alterations that occur in tumor cells are able to generate tumor-specific antigens (neoantigens), that are processed and presented on the tumor cells membrane, thus allowing to elicit the anti-tumor immune response after the activation of T cells (56, 57). The potential association of TMB with sensitivity to immunotherapy relies on the rationale that the production of neoantigens is increased in tumors with high TMB,

therefore boosting the response of the immune system (58). The role of TMB as a stratification marker to predict the response to anti-PD1/PD-L1 immune agents has been investigated in several tumor settings, mainly NSCLC and melanoma, showing promising yet still not conclusive results (59-61). In the specific setting of GC/GEJC, the effect of TMB to predict the response to pembrolizumab was explored in the negative second-line KEYNOTE-061 trial. The tissue TMB resulted to be statistically significantly associated with the clinical outcomes in the overall population treated with pembrolizumab, not stratified for MSI and PD-L1 status, but not with paclitaxel, and the results were maintained after adjusting for CPS. Nevertheless, after the exclusion of MSI-high patients, those endowed with the highest TMB (> 175 mut/exome), the effect was reduced, thus leaving unanswered questions about the effective role of TMB as a predictive marker (62).

FUTURE PERSPECTIVES

On the basis of the clinically significant results obtained by the recent pivotal clinical trials conducted on the topic of the integration of immunotherapy to the therapeutic algorithm of gastroesophageal carcinoma, the research is ongoing with the aim to optimizing the potential benefit by exploring novel combinations with immunotherapy.

First, the combination of pembrolizumab to trastuzumab was investigated in the setting of HER2 positive disease, by exploiting the potential synergy between the two drugs, since in preclinical models trastuzumab proved to upregulate PD-1 expression and induce an immune-sensitive gene expression signature, conversely pembrolizumab may augment HER2-specific T cell response and potentiate the activity of effector T cells. The biological proof was obtained first in a single-arm phase II study and afterwards confirmed in the first interim analysis of the randomized phase III Keynote-811 trial, where the addition of pembrolizumab to the standard first-line trastuzumab plus chemotherapy in HER2 positive advanced GC or GEJC conditioned a significant improvement in ORR with deeper and more durable responses (63, 64).

Second, in order to foster the immune response in generally poorly immunogenic tumors, such as MSS gastrointestinal cancers, the combination of ICI and anti-angiogenic agents was investigated, relying on the rationale that they could enhance immune activation besides remodeling tumor neoangiogenesis. In details, in preclinical studies, regorafenib showed to reduce tumor-associated macrophages and T regulatory cells. The phase Ib REGONIVO EPOC1603 study reported that the regorafenib plus nivolumab regimen is endowed with a manageable safety profile and encouraging antitumor activity in GC and colorectal cancer asian patients, to be potentially furtherly investigated in a larger population (65).

Finally, based on the results obtained in several tumor settings, the combination of the ICI pembrolizumab plus the multikinase inhibitor lenvatinib was explored in GC, on the rationale that lenvatinib proved to reduce the tumor-associated macrophages and increase the anti-tumor activity of PD-1 inhibitors thanks to the up-regulation of the interferon gamma signalling pathway. In details, an open-label single-arm phase II trial showed that this combination is endowed with promising anti-tumor activity and manageable safety profile in previously treated advanced GC patients (66). A randomized phase III Trial is ongoing to investigate the addition of the pembrolizumab-lenvatinib combo to the standard first-line chemotherapy in advanced GC patients (NCT04662710).

CONCLUSIONS

In conclusion, immunotherapy with ICIs entered the treatment scenario of GC/GEJC, since the anti-PD1 agents nivolumab and pembrolizumab proved to confer an improvement in the patients outcome. Nevertheless, the real magnitude of benefit of immunotherapy in this disease setting is under definition since the results of the landmark studies conducted so far showed that the selection of patients according to the predictive biomarkers of response to ICIs plays a key role in order to maximize the therapeutic efficacy. The next future will provide clinicians further data both on the definition of the optimal therapeutic algorithm in the different patients populations and on the investigation of combination regimens between chemotherapy, immune agents and possibly targeted therapies.

ETHICS

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Monica Niger received travel expenses from Celgene, speaker honorarium from Accademia della Medicina and consultant honoraria from EMD Serono, Basilea Pharmaceutica, Incyte and MSD Italia; Federica Morano received honoraria from Servier; Filippo Pietrantonio received honoraria from Amgen, Sanofi, Bayer, Servier, Merck-Serono, Lilly, MSD, Astrazeneca and research grants from Bristol-Myers Squibb and Astrazeneca; Maria Di Bartolomeo served on advisory board fo Myland Italy, received research grant from Lilly, payments for lectures including service on speakers bureaus from BMS, MSD, Lilly. The remaining authors declare no conflicts of interest.

Availability of data and materials

The data underlying this article can be shared just before a reasonable request to the corresponding author.

Authors' contribution

All the authors contributed equally to conception, data collection, analysis and writing of this paper.

Ethical approval

N/A.

Consent to participate

N/A.

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REVIEW

CAR-T CELL THERAPY. A NEW MILESTONE IN THE TREATMENT OF B-CELL LYMPHOMAS

M. E. Nizzoli[1,2], A. Bavieri[1,3], S. Luminari[1,4]

[1] Hematology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy
[2] Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Modena, Italy
[3] Resident in Hematology, University of Modena and Reggio Emilia, Reggio Emilia, Italy
[4] CHIMOMO Department, University of Modena and Reggio Emilia, Reggio Emilia, Italy

CORRESPONDING AUTHOR:

Stefano Luminari
Full Professor of Medical Oncology
Hematology Unit, Azienda USL-IRCCS di Reggio Emilia
viale Risorgimento 80
42123 Reggio Emilia, Italy
E-mail: sluminari@unimore.it
ORCID: 0000-0001-8446-2285

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ABSTRACT

The outcome of patients with malignant lymphoma has significantly improved over the last decades. Major contributions have come from an increased knowledge of the disease, from its better classification, and from relevant advances in treatment. Novel important therapies have been added to the existing approach, making it possible to improve direct cancer cell killing. Further, these new therapies also support the immune system to act against the tumor, opening the era of immuno-oncology (IO). In the field of IO, chimeric antigen receptor (CAR)-T-cell therapies represent one new effective approach that has so far produced unprecedented results in the treatment of lymphomas. Four CAR-T products are available to treat relapsed refractory patients with diffuse large B-cell lymphoma (DLBCL), transformed indolent lymphoma, primary mediastinal B-cell lymphoma, and mantle cell lymphoma. Several clinical trials are currently recruiting patients to evaluate the addition of novel indications for CAR-T, to anticipate the use of CAR-T to earlier lines of treatment for DLBCL patients, and to explore combination therapies. Moreover, the CAR approach is investigated using cells other than T lymphocytes to improve feasibility and reduce the toxicity of therapy. In this review we describe the state of the art of clinical research and real-world data on the use of CAR-T, which likely represents a new milestone in the treatment of malignant lymphomas.

KEY WORDS

CAR-T; adoptive cell therapy; diffuse large B-cell lymphoma; mantle cell lymphoma; follicular lymphoma.

IMPACT STATEMENT

Clinical trials and real-world data confirm CAR-T cell therapy as a revolutionary treatment for patients with diffuse large B-cell lymphoma.

INTRODUCTION

The recent history of malignant lymphomas has been marked by a few significant milestones that have made it possible to achieve the high cure rates observed in many of the cases, confirming this group of neoplasms as one of the most curable cancers in humans. A first milestone was the introduction in the 1990s of a robust integration of pathology, immune-morphology, and molecular biology in the initial diagnosis, which was incorporated in the WHO classification of malignant hematologic malignancies (1). A second milestone was achieved with the introduction of monoclonal antibodies, and in particular of the anti-CD20 agents, which permitted the identification of novel treatment paradigms based on the use of combined immunochemotherapy programs, mainly for B-cell lymphomas, which were all associated with improved efficacy compared to the old standard chemotherapy (2-4). A third milestone was the improvement of non-invasive techniques, such as 18FDG-PET, which achieve high accuracy in the identification of the disease at different timepoints and which contributed to refining staging and response criteria, thus providing useful details for treatment personalization (5).

The current recommended treatment options obtain high response rates in around 70% of the patients with an aggressive lymphoma, such as diffuse large B-cell lymphomas (DLBCL), and mantle cell lymphomas (MCL) and in up to 90% of subjects with more indolent subtypes, such as follicular lymphomas (FL). The use of immunochemotherapy has also improved patient survival, with approximately 60% of patients with DLBCL (6) being cured and unprecedented 5-year overall survival (OS) rates of around 70-80% for other subtypes like MCL or FL (7). Although significant improvements have been achieved in most of the malignant lymphomas, there remain important unmet clinical needs that are currently challenging the entire scientific community in its search for a solution. Among current challenges, how to manage patients with refectory or relapsed disease after standard immunochemotherapy is one of the most difficult treatment decisions. This is particularly true for patients with DLBCL, who experience a dismal survival when they fail a first conventional salvage therapy (8), but this is also true for other lymphoma subtypes for which available treatment options are rapidly exhausted after second or third relapse mainly due to the lack of active agents. In this setting of very high-risk patients with no active conventional options, the use of cellular therapy is emerging as a promising approach with positive results in hard-to-treat patients. Chimeric antigen receptor (CAR) T-cell therapies have proved effective and are currently registered for the treatment of DLBCL patients who relapse after high dose salvage therapy and will soon be registered for the treatment of high risk relapsed patients with MCL or FL as well. The possibility of achieving a cure in a significant proportion of patients lacking other therapeutic choices has shed light on this novel treatment modality, which merits becoming a new milestone in the treatment of malignant lymphomas and which may represent a significant improvement in the future treatment of other cancers. In this review we describe the main characteristics of CAR-T cell therapies and the main results achieved in malignant lymphomas. We also discuss the future development of CAR-T use in lymphomas.

CAR-Ts AND CAR PRODUCTS

CAR-T therapies, one of the most advanced tools of cellular-based cancer treatment, were developed thanks to the landmark experiments that defined the structure/function of the T-cell receptor (TCR) and evaluated adoptive cell therapies with tumor-infiltrating lymphocytes. The first genetically engineered T cell expressing a 1st generation CAR, which contained the variable antigen-recognition domains of an antibody linked to the constant transmembrane and intracellular CD3-zeta signaling domains of a TCR, was published in 1987 by

Yoshihisa Kuwana *et al*. (9). However it took nearly 20 years to successfully move the first CAR-T from the bench to the bedside and to improve transduction efficiency, CAR-T activation, and expansion and CAR construct optimization. The clinical development of CAR-T quickly moved to a 2nd generation CAR, which contains the addition of either a CD28 or 41BB intracellular co-stimulatory domain to augment CD3-zeta-mediated intracellular signaling and optimize T cell activation (10). In 2017, the pivotal ZUMA-1 trial evaluating axicabtagene ciloleucel, the 2nd generation CD19-targeted CAR-T cell product, demonstrated the remarkable efficacy of CD19 CAR-T cell therapies in relapsed refractory DLBCL and led to the first US FDA approval of a CAR-T therapy in this setting (11). Since then, additional 2nd generation CD19 CAR-T products for B-cell lymphoma, including tisagenlecleucel (12), brexucabtagene autoleucel (13), and lisocabtagene maraleucel (14) , have become available (**table I**).

Despite the high activity observed for CD19 CAR-T therapies in the pivotal studies, this treatment is associated with a unique safety profile, with potentially life-threatening toxicities including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) (15). CRS is associated with elevated serum levels of pro-inflammatory cytokines, including interferon gamma (IFN-gamma), TNF, IL-6, and IL-10, which contribute to a systemic hyperinflammatory syndrome characterized by fever, hypotension, and hypoxemic respiratory failure (16). The pathophysiologic mechanism of ICANS is likely related to an underlying endothelial dysfunction, leading to a leakage of elevated serum cytokine levels across the blood–brain barrier, thereby causing an inflammatory encephalopathy (17, 18). Considered together, the production of the CAR-T and the management of the patient during CAR-T therapy represent important challenges for clinical management.

CAR-T CELL STUDIES IN LYMPHOMAS

Phase II studies are available that describe the clinical activity and safety profile of the four CAR-T compounds.

The efficacy of axi-cel was demonstrated in the ZUMA-1 trial, published in 2017 and updated in 2019 (11, 19). The trial enrolled 111 patients with relapsed/refractory (r/r) DLBCL, primary mediastinal B-cell lymphoma (PMBCL), and transformed follicular lymphoma (tFL); of the enrolled patients, 101 received axi-cel infusion and 108 were included in the final evaluation. Bridging therapy was not allowed. The overall response rate (ORR) was 82%, with a complete response (CR) rate of 54%. Most of the responses were observed within six months from infusion, and the probability of response was correlated with CAR-T expansion within the first 28 days. The most recent study update, with 27.1 months of follow-up, demonstrated a median duration of response (DOR) of 11.1 months for all patient, with median DOR not reached for CR patients. Similarly, median OS was not reached, while median PFS was 5.9 months.

Regarding the safety profile, axi-cel treatment was associated with adverse events (AE) of any grade in 95% of the patients, hematologic events being the most frequent (neutropenia 78%, anemia 43%, and thrombocytopenia 38%). Ninety-three percent and 64% of the patients experienced CRS and ICANS of any grade, respectively. Severe CRS and ICANS occurred in 13% and 28%, respectively. Overall, three out of 44 deaths were referred as non-relapse events. Tocilizumab was used in 43% of patients with CRS and/or ICANS; corticosteroids were used in 27% of the entire cohort of patients. The efficacy of axi-cel was also demonstrated for patients with indolent lymphomas who were relapsed or refractory to at least two prior lines of therapy. As of 3 December 2020, 146 patients (124 FL; 22 MZL) received axi-cel. Patients had a median of three prior lines of therapy. With a median follow-up of 17.5 months, the ORR was 92%, with a 76% CR rate. In patients with FL (n = 84), the ORR was 94% (80% CR rate). The medians for DOR, PFS, and OS were not reached. The safety profile of the study was similar to that observed in the ZUMA1 trial (20).

The efficacy of tisa-cel was demonstrated in the JULIET trial initially published in 2019 (12) and was updated in 2020 and again in 2021 (12, 21). The trial enrolled 165 patients with DLBCL, tFL, and high-grade B-cell lymphoma (HGBCL) refractory or relapsed after two or more lines of therapy; 111 patients received tisa-cel infusion, and 93 were included in the final evaluation. The study allowed bridging therapy, which was used in 92% of the cases. The overall ORR was 52%, with a CR rate of 40% The most recent update of the study, with 60.7 months of follow-up, showed an ORR of 58%, with a CRR of 46%, and a median DOR of 61.4 months in patients with DLBCL. Regarding the safety profile, tisa-cel treatment was associated with grade 3-4 AE in 85% of the patients,

	AXICABTAGENE CILOLEUCEL	TISAGENLECLEUCEL	LISOCABTAGENE MARALEUCEL
Pivotal Trial	ZUMA-1(11) Phase I/II	JULIET (12) Phase II	TRANSCEND(14) Phase II
CAR Construct	α CD19 2nd gen, CD28	α CD19 2nd gen, 41BB	α CD19 2nd gen, 41BB
Leukapheresis	Fresh product direct to manufacturing (within US)	Cryopreserved product (could be stored before manufacturing)	Fresh product direct to manufacturing (within US)
Study Population	111 enrolled; 101 dosed 76% DLBCL; 16% tFL; 8% PMBCL 79% refractory 21% post-ASCT	165 enrolled; 111 dosed 80% DLBCL; 18% tFL 54% refractory 49% post-ASCT	344 enrolled, 269 dosed 51% DLBCL, 13% HGBCL, 6% PMBCL, 1% FL grade 3b 67% refractory 35% post-ASCT
CNS disease	No history of, or active, CNS disease allowed	No active CNS disease allowed	Secondary CNS involvement allowed
Patients receiving bridging therapy	Not allowed	92%	59%
Lymphodepleting Chemo	Flu 30 mg/m2 and Cy 500 mg/m2 on Days -5, -4, and -3	Flu 25 mg/m2 and Cy 250 mg/m2 on Days -5,-4, and -3 Bendamustine 90 mg/m2 daily for 2 days	Flu 30 mg/m2 and Cy 300 mg/m2 X 3 days, 2–7 days before infusion
CAR-T Dose	2.0 X 106 CAR-T cells/kg If > 100 kg, max. 2.0 X 108 CAR-T cells	Median, 3 x 108 CAR-T cells Range, 0.1-6.0 X 108 cells	DL1: 50 x 106 CAR-T cells (n = 45) DL1: 100 x 106 CAR-T cells (n = 183) DL3: 150 x 106 CAR-T cells (n = 41) (CD4:CD8 in 1:1 ratio)
Prior anti-CD19 therapy	Not allowed	Not allowed	Allowed, if CD19+ tumor present
Efficacy	OR: 82% CR: 54% Med. DOR: 11.1 mo. Med. PFS: 5.9 mo. OS at 18 mo.: 52%	OR: 52% CR: 40% Med. DOR: NR at 17 mo. Med. OS: 11.1 months	OR: 61% CR: 44% Med. DOR: NR at 12 mo. Med. PFS: 6.8 mo. Med. OS: 21.1 mo.
Safety	CRS: All grades: 93% ≥ Grade 3: 13% Neurotoxicity: All grades: 64% ≥ Grade 3: 28% Grade 5 AEs: 6%	CRS: All Grades: 58% ≥ Grade 3: 22% Neurotoxicity: ll grades: 21% ≥ Grade 3: 12% Grade 5 AEs: 3%	CRS: All Grade: 42% ≥ Grade 3: 2% Neurotoxicity: All grades: 30% ≥ Grade 3: 10% Grade 5 AEs: 0%

Table I. *Characteristics of available CAR-T products for the treatment of DLBCL.*

US: United States; FDA: Food and Drug Administration; gen: generation; DLBCL: diffuse large B-cell lymphoma; tFL: transformed follicular lymphoma; ALL: acute lymphoblastic leukemia; PMBCL: primary mediastinal B-cell lymphoma; MCL: mantle cell lymphoma; post-ASCT: post-autologous stem cell transplantation; chemo: chemotherapy; Flu: fludarabine; Cy: Cyclophosphamide; CAR-T: chimeric antigen T-cell; yrs: years; kg: kilogram; max: maximum; DL: dose level; CNS, central nervous system; OR: overall response; CR: complete response; Med.: median; DOR: duration of response; PFS: progression-free survival; OS: overall survival; mo.: months; CRS: cytokine release syndrome; AE: adverse event.

hematologic events being the most frequent (neutropenia 34%, anemia 48%, and thrombocytopenia 33%). Fifty-eight percent and 21% of the patients experienced CRS and ICANS of any grade, respectively. Severe CRS and ICANS occurred in 22% and 12%, respectively. No death was recorded as a non-relapse event. Tocilizumab was used in 14% of patients with CRS and/or ICANS; corticosteroids were used in 10% of the patients.

In the ELARA phase II study the activity of tisa-cel

was demonstrated in r/r FL within 6 months after second-/later-line therapy (22). As of May 26, 2020, 122 pts had been screened, 98 were enrolled, 97 received tisa-cel (median follow-up: 6.5 months), and 52 were evaluable for efficacy (median follow-up: 9.9 months). The median number of prior lines of therapy was four. CRR was 65.4% in the intention-to-treat (ITT) population and ORR was 82.7%. Median DOR, PFS, OS, and time to next anti-lymphoma treatment were not reached. Grade ≥ 3 AE were observed in 69% of patients, with a similar toxicity profile to that of the Juliet trial.

The efficacy of liso-cel was demonstrated in the TRANSCEND trial, published in 2020 (14). Differently from the other products, liso-cel is the only agent with a fixed 1:1 ratio of CD4/CD8 transduced and infused T cells. The study enrolled 344 patients with DLBCL, tFL, HGBCL, and FL grade 3B refractory or relapsed after two or more lines of therapy; 269 patients received liso-cel infusion, and 256 were included in the final evaluation. The study allowed bridging therapy, which was used in 59% of the cases. The overall ORR was 73%, with a CR rate of 53%. With 18.8 months of follow-up, the median DOR was not reached; median PFS and OS were 6.8 and 21.1 months, respectively.

Regarding the safety profile, liso-cel treatment was associated with AE of grade 3 or higher in 79% of the patients, hematologic events being the most frequent (neutropenia 60%, anemia 37%, and thrombocytopenia 27%). Forty-two percent and 30% of the patients experienced CRS and ICANS of any grade, respectively. Severe CRS and ICANS occurred in 2% and 10%, respectively; however, seven patients (3%) experienced non-relapse mortality. Tocilizumab or glucocorticoids were used in 20% of patients. Overall, no correlation was observed in the ZUMA-1, JULIET, and TRANSCEND trials between efficacy outcomes and age, DLBCL cell of origin, prior therapies, or use of steroids or tocilizumab. Nonetheless, high baseline tumor burden and high baseline pro-inflammatory markers were associated with lower treatment efficacy.

The efficacy of brexu-cel for the treatment of relapsed refractory mantle cell lymphoma was demonstrated in the ZUMA-2 trial, published in 2020 (13). The trial enrolled 74 patients with MCL refractory or relapsed after two or more lines of therapy, of whom 68 received brexu-cel infusion, and 60 were included in the final evaluation. Based on the intention-to-treat analysis, ORR was 85% and CRR was 59%. With 12.3 months of follow-up, 57% of the patients remained in remission, with an 83% 1-year OS and with a 1-year PFS of 61%. An ongoing confirmatory study is currently recruiting patients (NCT04880434).

Regarding the safety profile, brexu-cel was associated with AE of grade ≥ 3 in 99% of the patients, hematologic events being the most frequent (neutropenia 34%, anemia 48%, and thrombocytopenia 33%). Ninety-one percent and 63% of the patients experienced CRS and ICANS of any grade, respectively. Severe CRS and ICANS occurred in 15% and 31%, respectively. Tocilizumab was used in 59% of patients with CRS and/or ICANS; corticosteroids were used in 38% of the patients.

REAL-WORLD STUDIES ON CAR-T

The results of the above-mentioned pivotal trials led to the FDA's approval of the three products for the treatment of adult patients with r/r DLCBL, tFL, HGBCL, PMBCL (axi-cel and liso-cel only), and FL grade 3b (liso-cel only). The European Medical Agency (EMA) has so far approved only axi-cel and tisa-cel, with the same indications as in the US. More recently, the FDA approved the use of brexu-cel for the treatment of relapsed refractory MCL and axi-cel for the treatment of r/r FL.

Since FDA approval, several investigators have reported real-world studies (RWS) on the approved indications of CAR-T cell therapies in patients with aggressive lymphomas both in the US and in Europe. (see **table II** and **table III**).

A consortium of 17 institutions in the United States, the US Lymphoma CAR-T Consortium, performed a retrospective analysis evaluating the clinical outcomes of 298 patients treated with standard-of-care (SOC) axi-cel for r/r DLBCL (23). Patients had a median age of 60 years (range, 21-83 years). This included patients with poor PS, ECOG score 2-4 (19.5%), disease stage III-IV (82.4%), and international prognostic index (IPI) score 3-5 (54.4%). In the real world, axi-cel was used in patients with DLBCL (68.1%), PMBCL (6.4%), and tFL (25.5%). Of these, 22.8% had double- or triple-hit lymphoma, and 37.4% were double expressors.

Over half of the patients (53%) received bridging therapy (BT) of any kind showed worse OS than those who did not require BT. The poorer outcome associated with BT may be a result of pretreatment factors rather than the result of the BT alone. An interesting observation was made for patients who

	AXICABTAGENE CILOLEUCEL			TISAGENLECLEUCEL		
	Nastoupil *et al*. (23) (N = 298)	Jacobson *et al*. (24) CIBMTR (N = 1001)	Riedell *et al*. (26) (N = 149)	Pasquini *et al*. (25) CIBMTR (N = 155)	Riedell *et al*. (26) (N = 75)	Iacoboni *et al*. (30) (N = 91)
Histology, %						
DLBCL	68	-	86	-	94	73
tFL	26	28	-	27	-	23
PMBCL	6	-	-	-	-	-
Other	0	-	-	-	-	4
Median age, years (range)	60 (21-83)	62 (-)	58 (18-85)	65 (18-89)	67 (36-88)	60 (52–67)
Patients ≥ 65 years, %	52[a]	37	-	53	-	31
HGBCL/double/triple hit, %	23	14	-	11	-	15
Refractory/resistant to last line of therapy, %	42	62	-	-	-	29
ECOG PS, % 0-1	80	83	86	83	94	88
Previous autoSCT, %	33	29	29	26	23	39
Previous lines of therapy, median (range)	3 (2-11)	-	3 (2-11)	4 (0-11)	4 (2-9)	3(2-4)
≥ 3, %	75	-	-	-	-	28[b]
Received bridging therapy, %	53[c]	-	61	-	72	87
CR - PR, %	64 - 18	53 - 17	43 -	40 - 22	44 -	32 - 28
Median follow-up, mo.	12.9	12	-	11.9	-	14,1

Tables II. *Comparison of real-world data between axicabtagene ciloleucel and tisagenlecleucel.*
[a] Patients ≥ 60; [b] > 3 prior lines of therapy ; [c] bridging therapy included chemotherapy (54%), steroids only (23%), radiation therapy (12%), and targeted regimens (10%).

received radiotherapy as BT, which may result in improved PFS when compared with systemic therapy in select patients. Despite differences in the baseline characteristics of patients prescribed with SOC axi-cel, similar rates of toxicities were observed in comparison to ZUMA-1; CRS of any grade occurred in 91% of patients; 7% developed grade 3 or higher CRS, and one patient died as a result of HLH. Neurotoxicity occurred in 69% of patients, with grade 3 or higher occurring in 31%. One patient developed grade 5 cerebral edema. Tocilizumab and corticosteroids were given to 62% and 54% of patients, respectively, for CRS, neurotoxicity, or both.

Two real life studies were generated from the database of the Center for International Bone Marrow Transplant Research (CIBMTR). A first study was published on 1001 patients treated with axi-cel and observed for a median follow-up of 12 months (24). The median age of treated patients was 62 years, with 37% aged 65 or older. Twenty-eight percent had transformed lymphoma, and 14% high-grade lymphoma. The best ORR was 70% (CR 53%). The ORR, CR, 12-month PFS, and OS were 78% *vs* 66%, 60% *vs* 48%, 55% (95% CI, 48-62%) *vs* 40% (95% CI, 37-44%), and 70% (95% CI, 63-76%) *vs* 54% (95% CI, 50-58%), for chemosensitive and chemoresistant disease, respectively. The incidence of CRS grades ≥ 3 according to American Society for Transplantation and Cellular Therapy (ASTCT) Consensus Grading was 13%. ICANS were reported in 57% patients, with 26% of grade 3 or higher.

A second study was published on 155 patients treated with tisa-cel, reporting ORR and CR rates of 61.8% and 39.5%, respectively, which were very similar to those reported in the JULIET study (25). The median age was 65 years (range 18-89); 17 patients (11%) had double- or triple-hit features, and 27% had tFL. Any grade CRS occurred in 45%, grade 3 or higher occurred in 4.5%. Any grade ICANS occurred in 18%, grade 3 or higher occurred in 5.1%. The ORR was 62%, including the 40% achieving CR. Tocilizumab and corticosteroids were adminis-

AXICABTAGENE CILOLEUCEL + TISAGENLECLEUCEL				
	Chiappella *et al.* (28) SIE registry N = 113	Ghafouri *et al*. (45). N = 53	Le Gouill (29), DESCAR-T registry N = 550[c]	Dreger *et al.* (27) N = 267
Infused axi, n	59	45	350	137
Infused tisa, n	54	8	200	130
Histology, % DLBCL tFL PMBCL Other	 68 12 20 -	100 - - - -	 88 4 8 -	-
Median age, years (range) Patients ≥ 65 years, %	53 (19-70) -	63 (18-82) 60[a]	63 (18-79) 44	-
HGBCL/double/triple hit, %	16	14	1.7	-
Previous autoSCT, %	29	9	21	-
Previous lines of therapy, median (range) ≥ 3, %	3(2-7)	3(1-6) 32[b]	3 (1-10)	-
Received bridging therapy, %	86	58	82	79%
Patients who received CAR-T cells, (%)	100	100	100	100
ORR: CR - PR, %	40 - 31	64 - 8	53 - 21.2	Axi: 40 – 37 Tisa: 25 - 22
Median follow up, months	6,9	15,2	6.5	6.7

Tables III. *Comparison of real-world data between axicabtagene ciloleucel and tisagenlecleucel.*

[a] ≥ 60 years old; [b] ≥ 4; [c] data about patients who underwent leukapheresis

CIBMTR: center for International Blood and Marrow Transplant Research; DLBCL: diffuse large B-cell lymphoma; tFL: transformed follicular lymphoma; PMBCL: primary mediastinal B-cell lymphoma; HGBCL: high-grade B-cell Lymphoma; ECOG PS: Eastern Cooperative Oncology Group performance status; autoSCT: autologous stem cell transplant; CAR-T: chimeric antigen T-Cell; ORR: objective response rate; CR: complete response; PR: partial response rate; SIE: Società Italiana di Ematologia.

tered in 43% and in 10%, respectively.

Riedell *et al.* reported on 149 patients treated with axi-cel and 75 treated with tisa-cel at eight academic medical centers in the United States (26). Most patients were DLBCL with favorable PS and a median age of 58 years for axi-cel and 67 for tisa-cel. At day 90, the CR rate was 39% both for axi-cel and tisa-cel. Real-world data do not currently exist for liso-cel owing to its very recent approval.

Authors from the German Register for Stem Cell Transplantation (DRST) presented a first risk factor analysis of standard-of-care (SOC) CAR-T cell therapies for DLBCL(27). A total of 267 patients were included, who received axi-cel (137) or tisa-cel (130) for treatment of DLBCL until December 2020. Compared to the approval trials, patients were at relatively higher risk. Both CRS and neurotoxicity were significantly more common after axi-cel than after tisa-cel. Overall and complete response rates to axi-cel and tisa-cel were 77% and 47% (p < 0.0001), and 40% and 25% (p = 0.013), respectively. With a median follow-up of 6.7 months, progression/relapse occurred in 52% and 72% patients after axi and tisa-cel, respectively (p = 0.0027). Other significant risk factors for PFS on univariable analysis were elevated LDH, need for bridging therapy, and > 3 pretreatment lines. The adverse impact of tisa-cel, LDH, and bridging on PFS remained significant after multivariable adjustment for confounders (HR 1.51 (95% CI, 1.12-2.04), 1.55 (1.1-2.18), and 1.66 (1.12-2.46), respectively).

The Italian Society of Hematology (SIE) reported the results of a prospective RWS on 113 patients who were infused with axi-cel (59) or tisa-cel (54). The median age was 53 years (19-70); 52% of patients had DLBCL, 16% high-grade HGBCL, 20% PMBCL, and 12% tFL. Bridging therapy was delivered to 86% of patients. The median follow-up for infused patients was 6.9 months. CRR was 40% and ORR 71%. For the evaluable patients, DOR was 58% at

12 months. No differences between axi-cel and tisa-cel were reported. Grade 3-4 CRS was observed in only 5% of patients and severe ICANS in 10%. No toxic deaths were recorded(28).

In a similar study, Le Gouill *et al*. performed a retrospective analysis of patients in France treated with axi-cel or tisa-cel between April 2018 and March 2021 (29). A total of 550 patients were identified who received axi-cel (350) or tisa-cel (200). The median age was 63 (range 18 -79); 482 patients had DLBCL and 21 PMBL. The median number of prior lines was three, and 21% of patients had a prior ASCT; 80.2% received a bridging therapy. The median time between the CAR-T order and its infusion was 50 days (range 43 to 60 days). Response was available in 419 infused patients. Best ORR was 70.2%. At day 30 after CAR-T (D30) cell infusion, 38% patients achieved CR and 27% achieved PR. Among CR patients at D30 (157), 61% remained in CR at D90. The median follow-up was 7.4 months. The median OS calculated from time of CAR-T infusion was 12.7 months.

Iacoboni *et al*. reported the real-world experience with tisagenlecleucel in ten Spanish institutions (30). Of the 91 patients who underwent leukapheresis, 82% received tisa-cel therapy. The median age was 60 years; 58% of patients had DLBCL, 23% had tFL, and 15% had HGBCL; 87% received bridging therapy before infusion. The median time from apheresis to infusion was 53 days. The median follow-up from CAR-T cell infusion was 14.1 months. ORR and CR were 60% and 32%, respectively. Among the infused patients, 15% developed any grade of ICANS. Tocilizumab and steroids were administered to 32% and 21% of patients, respectively.

ONGOING TRIALS OF CD19 CAR-T CELL

The results of the phase II trials have paved the way for a wide range of studies aimed at four different main goals: increasing treatment safety, improving outcomes in the already addressed population, evaluating the potentialities of CAR-T cell therapies in the second line of treatment, and investigating CAR-T cells potentialities in other categories of patients (**table IV**).

Regarding the first goal, new strategies are currently being evaluated to lower the incidence and severity of CRS and ICANS through the use of JAK1 inhibitors such as itacitinib (NCT04071366), interleukin receptor antagonists (NCT04150913), or by introducing granulocyte-macrophage colony-stimulating factor antagonists such as lenzilumab (ZUMA-19, NCT04314843).

Regarding the second, the improvement of CAR-T cell efficacy in the relapsed/refractory DLBCL setting after second-line treatment is currently pursued through ongoing studies associating CAR-T with other drugs, such as acalabrutinib (NCT04257578), atezolizumab (ZUMA-6 study, NCT 02926833), and durvalumab or ibrutinib (PLATFORM trial, NCT03310619).

Three main studies are evaluating the efficacy of CAR-T cell in r/r DLBCL after first-line treatment by comparing cellular therapy to standard salvage chemotherapy (SOC) followed by autologous stem cell transplantation. These three studies, BELINDA, ZUMA-7, and TRANSFORM, which are evaluating tisa-cel, axi-cel and liso-cel, respectively, have shown promising preliminary results, with ZUMA-7 and TRANSFORM having reached the primary endpoint by demonstrating an event-free survival (EFS) advantage in the CAR-T cell arm. More precisely, ZUMA-7 trial randomized 359 patients in a 1:1 ratio between axi-cel and SOC, outlining an increased CR rate (65% *vs* 32%) and EFS (8.3 months *vs* 2 months) (31). Of note, bridging therapy was not permitted in accordance with the previous ZUMA-1 trial. Analogously, the TRANSFORM trial compared liso-cel with SOC, demonstrating advantages in the CAR-T cell arm in terms of CR rate (66% *vs* 39%, $p < 0.0001$) and EFS (10.1 months *vs* 2.3 months, $p < 0.0001$) (32). On the other hand, Belinda trial comparing tisa-cel with SOC failed to meet its primary endpoint (EFS). Among 322 randomized patients, ORR at week 12 was 46% in experimental arm and 43% in observational arm (33).

Regarding the fourth goal, several studies are investigating CAR-T cell therapies in different patient cohorts and different B-cell neoplasms. Among them, LYSARC (the Lymphoma Academic Research Organisation, NCT04531046) is evaluating axi-cel as a second-line therapy in unfit patients, while the BIANCA trial (NCT 03610724) is investigating tisa-cel in children and young adults with DLBCL. The ZUMA-12 trial (NCT03761056), another phase II study assessing axi-cel in high-risk DLBCL patients with suboptimal interim response to first-line therapy, showed promising preliminary results, with a CR rate of 80% and EFS not reached after a median follow-up of 15.9 months. Among trials that are investigating the efficacy of CAR-T cell therapy in other B-cell neoplasms, the TARMAC trial is evaluating a combination of tisa-cel and ibrutinib in

TRIAL NAME (NCT NUMBER)	INDICATION	DRUGS	PHASE	LINE OF THERAPY
ZUMA-12 (NCT03761056)	High risk DLBCL	Axi-cel	2	1st
NCT04531046	Transplant ineligible r/r aggressive B-cell NHL	Axi-cel	2	2nd
TIGER-CTL019 (NCT04161118)	Transplant ineligible r/r aggressive B-cell NHL	Tisa-cel	2	2nd
TRANSCENDWORLD (NCT03484702)	r/r aggressive B-cell lymphoma	Liso-cel	2	≥ 2nd
NCT04608487	r/r aggressive B-cell lymphoma with primary or secondary CNS involvement	Axi-cel	1	≥ 2nd
NCT04257578	B-cell NHL	Axi-cel + Acalabrutinib	1-2	≥ 3rd
ZUMA-6 (NCT02926833)	Refractory DLBCL	Axi-cel + Atezolizumab	1-2	≥ 3rd
ZUMA-19 (NCT04314843)	r/r DLBCL	Axi-cel + Lenzilumab	1-2	≥ 3rd
NCT05077527	r/r HIV-associated aggressive B-cell NHL	Axi-cel	1	≥ 3rd
PLATFORM (NCT03310619)	r/r DLBCL	Liso-cel + Durvalumab or CC-122	1-2	≥ 3rd
TRASCEND-OUTREACH-007 (NCT03744676)	r/r DLBCL or FL 3b	Liso-cel, outpatient setting	2	≥ 3rd
NCT03876028	r/r DLBCL	Tisa-cel + Ibrutinib	1b	≥ 3rd
TARMAC (NCT04234061)	r/r MCL	Tisa-cel + Ibrutinib	2	≥ 2nd
TRANSCEND FL (NCT04245839)	r/r FL (grades 1-3a) or MZL	Liso-cel	2	≥ 2nd
NCT03331198	r/r CLL or SLL	Liso-cel + Ibrutinib/ Venetoclax	1-2	≥ 3rd

Table IV. *Ongoing clinical trials exploring further applications of approved CAR-T cell products in adult B-cell lymphomas.*
DLBCL: diffuse large B-cell lymphoma; NHL: non-Hodgkin lymphoma; MCL: mantle cell lymphoma; MZL: marginal zone lymphoma; FL: follicular lymphoma; CLL: chronic lymphocytic leukemia; SLL: small lymphocyte lymphoma.

mantle cell lymphoma (NCT 04234061) and TRANSCEND FL (NCT04245839) is investigating CAR-T therapies in follicular lymphoma. Also, the TRANSCEND-CLL-044 is assessing CAR-T cell therapy in chronic lymphocytic leukemia/small lymphocytic lymphoma (NCT03331198).

FUTURE OF CAR TECHNOLOGY

The advent of CAR-T cell therapies has dramatically improved outcomes in the relapsed/refractory setting of several lymphoma subtypes. However, refractoriness to CAR-T treatment, relapse after initial response, therapy-related toxicities, and treatment costs represent relevant hurdles to overcome (34).

Therefore, several research fields are being developed towards different aims: to identify strategies to increase CD-19 CAR-T activity and persistence, to target new antigens in B-cell neoplasms, and to identify alternative platforms for CAR engineering. Overall, the optimization of CD-19 CAR-T function is mainly pursued in three different ways. First, through the co-administration of drugs capable of hindering immune escape such as programmed death protein 1 inhibitors (PORTIA trial, NCT03630159) or drugs able to stimulate T cell expansion (*e.g.*, interleukin-7 receptor agonists). Second, through the employment of CRISPR-Cas9 gene editing to e ndow CAR-T cells with the ability to counteract tumor-dependent immunosuppressive signals like TGF-β (35, 36). Third, through the modification of CAR construct by introducing additional co-stimulatory domains (3rd generation CAR-T cells) or additional endodomains capable of inducing stimulatory cytokines production (4th generation) (10).

Although CD19 represents an ideal target for CAR-T cells because it is expressed uniformly at high site density on B-cell malignancies, targeting a single antigen in cancer is fraught with the potential for antigen loss variants to emerge. Therefore, new targets are sought in order to hamper this phenomenon. Among them, CD20 and CD22 represent the most promising targets thanks to their uniform presence and persistence in B-lymphoid cells (37). Several phase I-II trials with CD20 or CD22 CAR-T cells are ongoing (*e.g.*, NCT03277729). Among other B-cell receptor targets, CAR-T cells directed against CD79a, CD37, and BAFF-R are currently under development.

The immune escape through antigen drop can also be overcome through multiple antigen targeting. Three different techniques are being tested in order to pursue this aim: 1) the co-administration of two or more CAR-T cell lines, with each line expressing a different antigen specificity; 2) the co-transduction of T-cells with two vectors encoding the two separate CARs (bi-specific CAR-T, NCT04007029); 3) T-cell engineering with only one vector encoding both CARs (bicistronic CAR-T) (38). The latter strategy may lead to a less expensive and more homogeneous product and is therefore regarded as a very promising approach.

The development of new CAR-T cell therapies with enhanced efficacy and wide target range will probably lead to a broader application in hematology-oncology. Manufacturing time and costs represent therefore a substantial limitation to the use of CAR-T, which must be overcome. In order to pursue this aim, several efforts are directed towards the identification of alternative vehicles for CAR engineering that can be manufactured and stored to be readily available, with reduced costs and waiting time. Allogeneic CAR-T cells represent one of the most promising approaches. T lymphocytes obtained from peripheral blood mononuclear cells from healthy donors, umbilical cord blood, or derived from induced pluripotent stem cells undergo a gene editing process able to confer resistance to host rejection and independence from major histocompatibility complex (MHC) for T cell activation, and are also able to avoid graft-versus-host reaction (GVHD) (39). These so-called "off-the-shelf" universal CAR-T (U-CAR-T)(40) are currently in the early phase of clinical development (*e.g.*, NCT04264039). Another promising CAR vehicle is represented by natural killer (NK) cells, which, unlike T cells, can kill transformed cells without the need for prior antigen priming and without MHC restriction. Moreover, allogeneic NK cells do not induce GVHD. NK cells can be derived from autologous or allogeneic sources and can be propagated *in vitro* (41-43). A first-in-human phase I-II trial employing CAR-NK cells in r/r B-cell malignancies, with promising results, has been published. A third source of alternative CAR vehicles is represented by cytokine-induced killer cells (CIK), immune effector cells featuring a mixed T and NK cell phenotype that can kill both in an MHC-dependent and -independent manner. This approach is currently being evaluated in B-cell acute lymphoblastic leukemia (44).

Lastly, other ongoing trials may influence the future of CAR-T cell therapies by broadening their use and lowering therapeutic costs. These trials focus on the development of CAR-T management regimens that may allow outpatient administration. Recruiting trials are available for liso-cel (TRASCEND-OUTREACH-007, NCT03744676) and axi-cel (NCT05108805).

CONCLUSIONS

In conclusion, the available data on the efficacy of CAR-T therapies and the numerous planned and recruiting clinical trials confirm this novel treatment modality as a new milestone for the treatment of lymphomas. The use of CAR-T has already made it possible to treat several patients around the world and to cure a significant proportion of subjects lacking other effective alternative options. Real-world data are reassuring about the possibility of moving a complex treatment modality from the bench of clinical trials to the bedside of our patients. The science of CAR-T, and more in general that of adoptive cell therapies, has gained momentum as one of the most promising approaches to the treatment of cancer in humans and will likely impact the near future of oncology.

ETHICS

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Conflict of interests

Prof Stefano Luminari has had a role as advisor for the following companies: Roche, Jannsen, Gilead/kite, BMS/Celgene, Regeneron, Genmab, and Abbvie.

Availability of data and materials

N/A.

Authors' contribution

All authors contributed to manuscript writing and approved the final version.

Ethical approval

N/A.

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REVIEW

COVID-19, ENVIRONMENT, CLINICOPATHOLOGIC FEATURES, LABORATORY FINDINGS AND DIAGNOSIS, TREATMENT, VACCINES, ANIMALS, AND CANCER

A. Amirkhani Namagerdi[1], F. Ciani[1], D. d'Angelo[1], L. Carangelo[2], F. Napolitano[1,3], L. Avallone[1]

[1] Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy
[2] Centro di Farmacovigilanza e di Farmacoepidemiologia di rilevanza regionale della regione Campania, Università degli studi della Campania Luigi Vanvitelli
[3] CEINGE - Biotecnologie Avanzate, Naples, Italy

CORRESPONDING AUTHOR:

Francesca Ciani
Department of Veterinary Medicine and Animal Production
University of Naples Federico II
via F. Delpino 1
80137 Naples, Italy
E-mail: ciani@unina.it
ORCID: 0000-0002-9188-6761

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ABSTRACT

SARS-CoV-2 (COVID-19) belongs to the same coronavirus group (Beta-coronavirus) as SARS and MERS viruses that caused two of the more severe epidemics in recent years. Horseshoe bats (genus Rhinolophus) have been identified as the natural reservoirs of SARS-related coronaviruses (CoVs) and the likely origin of SARS-CoV-2. The intermediate host is thought to be the pangolin. The purpose of this review is to draw attention to the relationship between COVID-19 and different malignancies, and to discuss the similarities in their pathogenesis, and the possible repurposing of cancer drugs for the treatment of COVID-19. Along with antiviral and anti-inflammatory drugs, several anti-cancer drugs can be potentially repurposed in the management of COVID-19. The pathogenesis of COVID-19 and cancer shares certain similarities, including inflammation, immunological dysregulation, and coagulopathy. Blood parameters in COVID-19 patients upon admission show lymphocytopenia, and elevated C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), and D-dimer levels in most of the patients. Currently, RT-PCR is the gold-standard laboratory test for COVID-19 confirmation in suspected cases.

KEY WORDS

COVID-19; SARS-CoV-2; cancer; environment; animals.

IMPACT STATEMENT

This review wants to address some aspects of COVID-19, such as environment, pathophysiology, laboratory findings, diagnosis, therapeutic and preventive treatment, role of different animals in transmission, with particular attention to cancer.

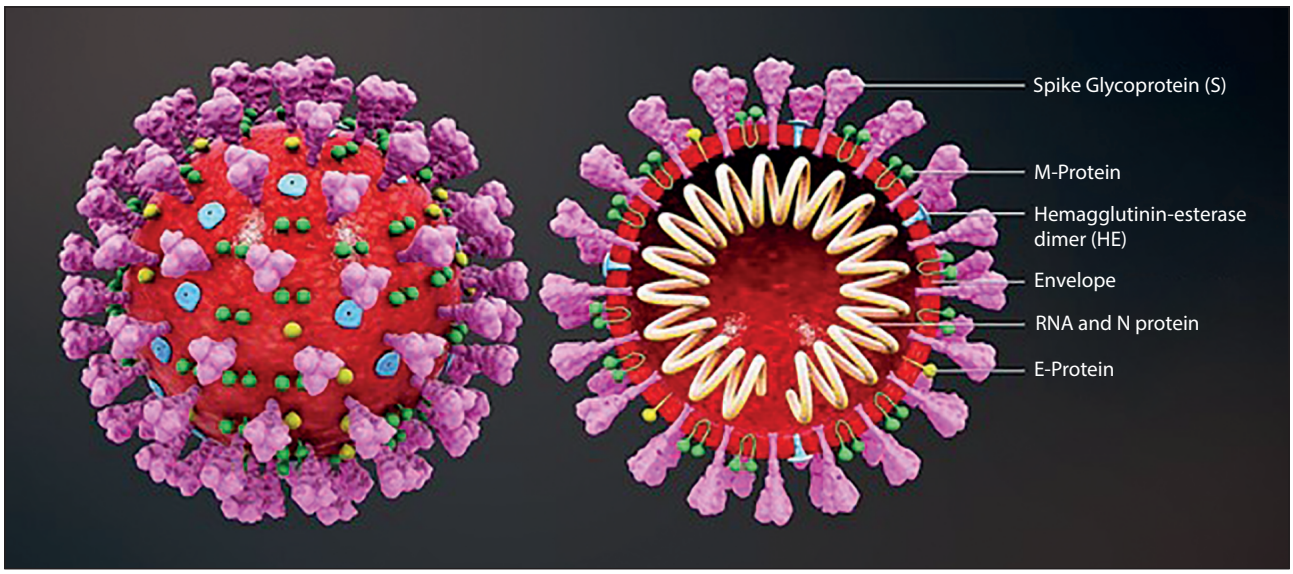
INTRODUCTION

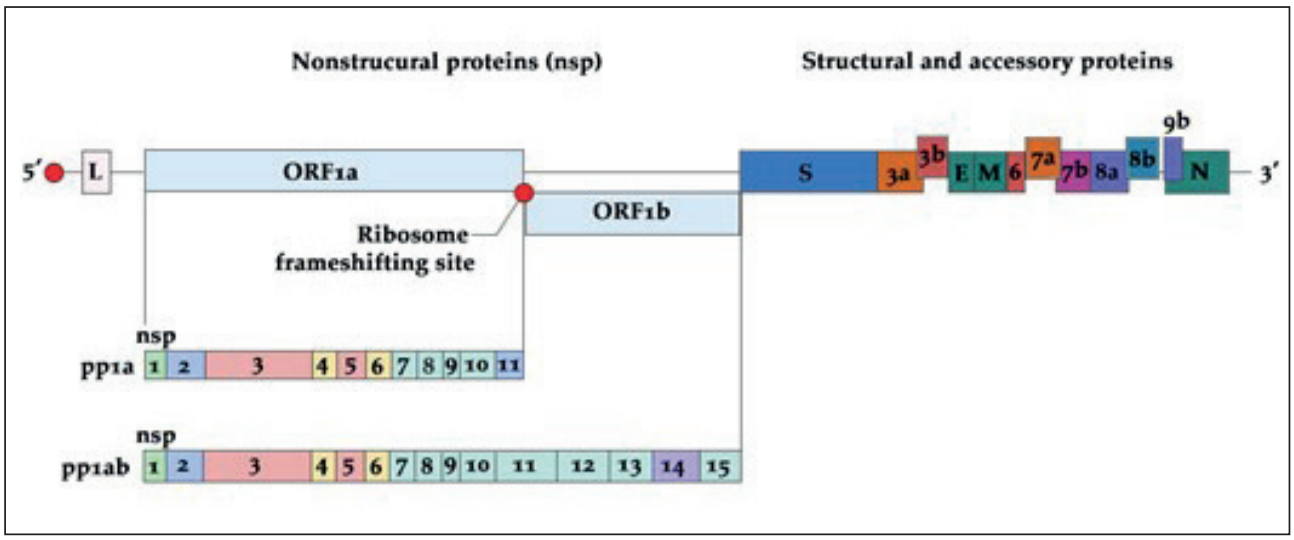
Coronaviruses are members of the *Coronaviridae* family in the Nidovirales order. The coronavirus family is divided into four subgroups: alpha (*α*), beta (*β*), gamma (*γ*), and delta (*δ*). Alpha- and beta-coronaviruses are found in mammals, particularly bats, while gamma- and delta-coronaviruses are found in pigs and birds (1). SARS-CoV-2 is a member of the same coronavirus family (Betacoronavirus) as the SARS and MERS viruses, which were responsible for two of the most devastating epidemics in recent years (2). Horseshoe bats (*genus Rhinolophus*) were discovered to be natural reservoirs of SARS-related CoVs and the likely source of SARS-CoV. Fruit bats (*Rousettus aegypticus*) can become infected and transfer the disease to other bats (3). Malayan pangolins have also been shown to have closely similar sequences (4). The inclusion of an intermediary, such as the pangolin, is suggested by the fact that SARS-CoV-2 was initially detected in Wuhan, China, far from where the horseshoe bat is found (5). These are enveloped viruses. The capsid is made up of the nucleocapsid protein N, which is surrounded by a membrane composed of three proteins: the membrane protein (M), the envelope protein (E), which are involved in the virus budding process, and the spike glycoprotein (S) (6). The SARS-CoV-2 virus is 50-200 nm in diameter and contains a + ssRNA genome of approximately 29.9 kb in length, making it the biggest known RNA virus. It has a 5'-cap structure and a 3'-poly-A-tail, and 14 putative open reading frames (ORFs) encoding 27 proteins (7). The SARS-CoV-2 genome has ten genes. The genes are organized in the following order: 50-replicase-S-E-M-N-30, with genes for accessory proteins inserted among structural genes (S, E, M, N). The polymerase gene, which has two overlapping open reading frames (ORFs), **figures 1 *a*** and ***b***, takes up around two-thirds of the total RNA(8). SARS-CoV-2 uses ACE2 as an attachment receptor and TMPRSS2 for spike protein priming, membrane fusion, and cell entry. TMPRSS2 cleaves ACE2 at Arginine 697-716 (9). The ACE2 gene is an escape gene that is found in the Xp22.2 region of the X chromosome. In principle, females get a double dose of ACE2, which might compensate for the loss of membrane ACE2 caused by SARS-CoV-2 (10). (**figures 1 *a*, *b***).

Quite recently, due to its importance, considerable attention has been paid to, and many publications have been released about the different features of COVID-19. The purpose of this review is to draw attention to the relationship between COVID-19 and different malignancies, and to discuss the similarities in their pathogenesis, and the possible repurposing of cancer drugs for the treatment of COVID-19. The remainder of the paper is organized into ten sections, starting with Section II, which discusses the role of the environment, and ending with Section X, which is devoted to cancer.

COVID-19 AND ENVIRONMENT

The origins of emerging infectious diseases (EIDs) are strongly linked to socioeconomic, environmental, and ecological factors. Changes in the manner and intensity of land use around the world are creating more dangerous interfaces between people, animals, and wildlife, which are a zoonotic disease reservoir (12). Because of the loss of habitat, animals are forced to move, where they may come into touch with other animals or people and spread germs (13). Humans and cattle are more likely to come into touch with wildlife, particularly in regions where forest cover has been reduced by more than 25% (14). Pathogen transmission from wild animals to domestic animals and humans, and vice versa, has resulted in major epidemics and pandemics around the world (14). Large livestock farms can also serve as a source for spillover of infections from animals to people (13). People are infected directly or indirectly by zoonotic viruses when they touch live primates, bats, and other wildlife (or their meat) or farm animals such as chickens and pigs (15). Live and dead wild animals come into contact with hunters, traders, customers, and everyone else involved in the wildlife trade at wildlife markets and in the legitimate and criminal wildlife trade (15). Air pollution, particularly NO2 and PM2.5 (particles with a diameter of less than 2.5 micrometers), may increase the susceptibility to infection and mortality from COVID-19 (16). PM2.5 and NO2 have a strong relationship with COVID-19 (17). Climate change is caused by carbon dioxide (CO2) emissions. Pandemic risk is increased by several of the core causes of climate change. For centuries, carbon dioxide persists in the atmosphere and oceans. CO2 emissions have fallen globally owing to coronavirus lockdown. According to scientists, this will be the largest reduction in manmade CO2 emissions since World War-I





Figures 1 ***a****. Schematic presentation of the SARS-CoV-2;* ***b****. its genome structure. SARS-CoV-2 has a spherical structure. The virus has an outer lipid envelope, covered with spike glycoprotein. The RNA genome has a replicase complex (comprised of ORF1a and ORF1b) at the 5'UTR. The ORF1a encodes for nsp1-nsp11, while ORF1b encodes for nsp1-nsp15. Four genes that encode for the Structural proteins: Spike gene, Envelope gene, Membrane gene, Nucleocapsid gene and a poly (A) tail at the 3'UTR. The accessory genes are distributed in between the structural genes. (****a****: credit to https://www.scientificanimations.com/wiki-images/;* ***b****: modified from (11)).*

(18). According to NASA researchers, ozone concentrations above the polar parts of the planet declined by roughly 240 Dobson units on March 12, 2020, compared to March 12, 2019. Low levels like these are extremely unusual, occurring just once every ten years or so (19).

PATHOPHYSIOLOGY

The spike glycoprotein-S enhances the virus's attachment to the angiotensin-converting enzyme 2 (ACE2) receptor and allows it to fuse with the host cell's membrane (7). There are two functional subunits in the S glycoprotein. The S1 subunit, which contains the RBD, is important for binding to the host cell receptor, while the S2 subunit is important for fusing of the viral and cellular membranes (20). SARS-CoV-2 then infects target cells by using serine proteases TMPRSS2 (transmembrane protease serine 2) for S protein priming (7). This protein is used for cell entrance by the influenza virus and the human coronaviruses HCoV-229E, MERS-CoV, SARS-CoV, and SARS-CoV-2 (COVID-19 virus) (21). Type 2 alveolar cells, nasal goblet cells, nasal ciliated cells, corneal cells, and intestinal epithelial cells are all likely SARS-CoV-2 host cells since they show high amounts of both ACE2 and TMPRSS2. SARS-CoV-2 appears to infect mononuclear phagocytes but not lymphocytes among

immune cells (22). SARS-CoV-2 infects pulmonary capillary endothelial cells in addition to epithelial cells, amplifying the inflammatory response and triggering an influx of monocytes and neutrophils (23). 15-30% of persons who are hospitalized with COVID-19 will develop COVID-19-associated acute respiratory distress syndrome (ARDS) (24). Patients with COVID-19-related ARDS who have decreased respiratory system compliance and elevated D-dimer concentrations have a high death risk (25). Two genetic susceptibility loci at Chr3p21.31 and Chr9q34.2 were discovered in the first genome-wide association study (GWAS) of severe COVID-19 with ARDS. The locus Chr3p21.31 spans the genes SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, XCR1, CCR1, and include several chemokine receptors (CCRs, CXCR6, and XCR1) that mediate chemokine signaling pathways for leukocyte chemotaxis and cause lung injury (26). The presence of high levels of ACE2 in the intestine makes the small bowel and colon particularly vulnerable to SARS-CoV-2 infection. According to the Human Protein Atlas database, the expression of ACE2 messenger RNA and protein in the gut is 100 times higher than in the lung (27). SARS-CoV-2 is largely spread from person to person through close contact (about 2 m) and aerosol respiratory droplets with a diameter of less than 5 μm in diameter (17). Longer exposure to an infected person (at least 15 minutes within 6 feet) and shorter exposures to symptomatic individuals are linked to a higher probability of transmission (28). Both TMPRSS2 and ACE2 are found in human corneal epithelial cells, implying that ocular surface cells could be viral entry sites as well (29). Another mechanism of transmission is contact surface spread. Aerosols may also be a cause of infection in humans outside of a laboratory setting, however it is unknown if this is a substantial cause of infection in humans (28). Viral shedding can begin 5 to 6 days before the first symptoms occur, and infectiousness can drop dramatically 8 days after the first symptoms occur (30). The median incubation period was calculated to be 5.1 days among 181 confirmed cases with known exposure and symptom start dates, and 97.5 percent of those who develop symptoms go through with within 11.5 days following infection (31). The most prevalent symptoms in hospitalized patients are fever (up to 90% of patients), dry cough (60%-86%), shortness of breath (53%-80%), fatigue (38%), nausea/vomiting or diarrhea (15%-39%), and myalgia (15%-44%). Non-classical symptoms, such as isolated gastrointestinal complaints, can also be present. In 64% to 80 of patients, olfactory and gustatory dysfunctions have been recorded (28). The underlying pathophysiology of the loss of these olfactory and gustatory perceptions has been linked to direct damage to the olfactory epithelium's supporting cells, the olfactory bulb, and altered olfactory neuron function, altered ACE2 signal transmission, and intensified gustatory particle degradation by sialic acid (32). Hypertension (56.6%), obesity (41.7%), and diabetes (33.8%) were the most common comorbidities in 5700 hospitalized patients, according to clinical research (33). Acute renal injury (9%), liver dysfunction (19%), bleeding and coagulation dysfunction (10%-25%), and septic shock (6%) are all possible complications for hospitalized patients (28). It is unclear why children are less likely to contract COVID-19. The following are some possible explanations: Children's immune responses are less vigorous (no cytokine storm), they have partial immunity from prior viral exposures, and they have lower rates of SARS-CoV-2 exposure. SARS-CoV-2 infection has recently been linked to a rare multisystem inflammatory illness similar to Kawasaki disease in children in Europe and North America (34). RNA viruses have a higher rate of mutation than DNA viruses. Coronaviruses, on the other hand, create fewer mutations than most RNA viruses because they encode an enzyme that corrects some replication errors (34). In the global pandemic, a SARS-CoV-2 variant with the Spike protein amino acid mutation D614G has become the most common type. The transition from D614 to G614 happened asynchronously in different parts of the world, starting with Europe, then North America and Oceania, and finally Asia (35). This dominant strain is ten times more infectious than Wuhan-1 strain. In both Denmark and the Netherlands, a mink-related variation Y453F has been discovered (36). Y453F is found in the RBD and is most likely a mink ACE2 adaptation, but it also boosts affinity for human ACE2 and replicates as well as the wildtype (37). In the United Kingdom, a SARS-CoV-2 variant B1.1.7 with a mutation Δ69/70 has spread fast (38). This variant accounted for around 28% of SARS-CoV-2 infection cases in England as of December 28, 2020, and population genetic models imply it is spreading 56 percent faster than other lineages (34). The E484K mutation can be found in different variants including, the South African (B.1.351), Brazilian (B.1.1.28), and UK B.1.1.7 var-

iants (39). The SARS-CoV-2 B.1.617 lineage was identified in October 2020 in India (40). The lineage includes three main subtypes (B1.617.1, B.1.617.2 and B.1.617.3), harbouring diverse Spike mutations in the N-terminal domain (NTD) and the receptor binding domain (RBD) which may increase their immune evasion potential. B.1.617.2, also termed variant Delta, is believed to spread faster than other variants. Sera from individuals having received one dose of Pfizer or AstraZeneca vaccines barely inhibited variant Delta. Administration of two doses generated a neutralizing response in 95% of individuals, with titers 3 to 5 fold lower against Delta than Alpha(B.1.1.7) (41). The Lambda (C.37) lineage was classified as a variant of interest (VOI) by the World Health Organization on June 15th, 2021. The C.37 variant, which lies within the B.1.1.1 lineage, has already been reported as highly prevalent in Peru and has also been identified in many countries across the Americas, Europe and Oceania (42). The Omicron variant, also known as B.1.1.529, is a novel extensively mutated SARSCoV2 variant that was identified as a variant of concern (VOC) by the World Health Organization on November 26, 2021 (43).

LABORATORY FINDINGS

The average range of laboratory abnormalities identified in COVID-19, according to a systematic evaluation of 19 studies involving 2874 patients included elevated serum C-reactive protein (increased in > 60% of patients), LDH (increased in approximately 50%-60%), alanine aminotransferase (elevated in approximately 25%), and aspartate aminotransferase (approximately 33%) (28). According to Huang *et al.* (44) a serum albumin level of < 35 g/L at presentation increased the risk of death in COVID19 by at least 6 times. According to Khourssaji *et al.* (45), blood parameters in COVID-19 patients upon admission indicated elevated C-reactive protein (CRP) (100%), ferritin (92%), LDH (80%), white blood cell (WBC) count (26%) with lymphocytopenia (52%) and eosinopenia (98%). Yao *et al.* (46) showed that D-dimer elevation (≥ 0.50 mg/L) was found in 74.6% (185/248) of the patients, and D-dimer level of > 2.14 mg/L predicted in-hospital mortality with a sensitivity of 88.2% and specificity of 71.3%. Al-Samkari *et al.* (47) described that in a multicenter retrospective study of 400 hospital-admitted COVID-19 patients, additional markers at initial presentation predictive of thrombosis during hospitalization included platelet count > 450 × 109/L, CRP > 100 mg/L, and erythrocyte sedimentation rate (ESR) > 40 mm/h. COVID-19-associated coagulopathy (CAC) has characteristics that are unique from bacterial sepsis-induced coagulopathy (SIC) and disseminated intravascular coagulation (DIC) (48). COVID-19-induced coagulopathy (CIC) is characterized by a significant increase in D-dimer and fibrin split products but little or no change in activated partial thromboplastin time and prothrombin time upon presentation (49). Neutrophil extracellular traps (NETs), including cell-free DNA, are higher in severe COVID-19 patients requiring mechanical ventilation, according to Zuo *et al.* (50), and have a substantial correlation with acute phase reactants such as CRP, D-dimer, and LDH. These NETs have the capacity to spread inflammation and microvascular thrombosis. The occurrence of the virus-induced "cytokine storm" has been related to mortality in COVID-19 patients (51). Guo *et al.* (52) showed that, D-dimer rises before the cytokine storm reflected by the IL-6 rise, implying that coagulopathy could operate as a signal to intensify a cytokine storm. COVID-19 infection appears to cause a worse cytokine storm, culminating in widespread micro- and macrovascular thrombosis and organ failure (49). Calprotectin, CRP, IL-1, IL-10, and tumor necrosis factor (TNF-) are all up to 200-fold higher than normal, while IL-6 can be up to 1000-fold higher than normal in recorded cases (26). In the event of SARS-CoV-2, higher innate immune system cytokine levels, such as IL-8 and IL18, are linked to greater severity in men. Females, on the other hand, had a lower severity in line with a larger T-cell activation (10). Within 3 weeks of the onset of symptoms, patients with Covid-19 develop kidney dysfunction, primarily acute kidney injury (AKI), hematuria, and proteinuria. The pathogenesis of AKI can be linked to COVID-specific mechanisms (direct viral entry, unbalanced RAS activation, virally induced proinflammatory cytokines, and thrombotic state) as well as nonspecific pathways (right heart failure, hypovolemia, nosocomial sepsis, nephrotoxic drugs, high PEEP in cases demanding mechanical ventilation and hemodynamic changes) (53). The GI signs seen in COVID-19 are caused by SARS-CoV-2 infection of intestinal enterocytes, which leads to ileum and colon dysfunction. According to multiple studies, people with severe COVID-19 have increased liver enzymes and a higher rate of

liver injury. Patients with abnormal liver function tests, particularly raised alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were also more likely to develop severe pneumonia (54). Wang *et al.* (55) found that, the rate of pancreatic damage was not actually low (17%) among the 52 patients with COVID-19 pneumonia. COVID-19 appears to exacerbate diabetic problems, most likely due to viral-induced pancreatic dysfunction, along with immunological dysregulation, vasculopathy, and coagulopathy (56).

DIAGNOSIS

Antibody tests use lateral flow assays to quickly identify antigens (spike, membrane, or nucleocapsid proteins) or antibodies for COVID-19. Rapid diagnostic tests (RDT), enzyme-linked immunosorbent assays (ELISA), neutralization assays, and chemiluminescent immunoassays are the four main types of antibody tests (57). The companies have centered their efforts on developing ELISA kits for detecting serum antibodies against two S protein domains (S1 and S2). In detecting antibodies from mildly infected COVID-19 patients, the RBD and N ELISA tests were found to be more sensitive than the S1 ELISA test (58). When compared to other testing methods, molecular diagnostic procedures are more appropriate since they target the pathogen's genome or proteome, making them more specific and precise (59). Currently, the gold-standard laboratory test for COVID-19 confirmation in suspected patients is RT-PCR (60). Eight of 13 studies evaluating SARS-CoV-2 viral load in serial upper respiratory tract samples showed peak viral loads calculated based on cycle threshold values within the first week of symptom onset (61). Among 1070 specimens collected from 205 patients with COVID-19, bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%) by RT-qPCR testing followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%), fibro-bronchoscope brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feces (44 of 153; 29%), and blood (3 of 307; 1%). None of the 72 urine specimens tested positive (62). Droplet digital PCR (ddPCR) is the most extensively utilized method among different partitioning methods (microwell plates, capillaries, oil emulsion, miniaturized chambers) (63). In the detection of low-viral load samples, digital PCR (dPCR) surpasses RT-qPCR and can be used as a complement (64). Notomi *et al.*[65] created a new method called loop-mediated isothermal amplification (LAMP) in the year 2000, which amplifies DNA with high specificity, efficiency, and rate under isothermal conditions. They used a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. Rabe *et al.* (66) recently established a sensitive (RT-LAMP) assay compatible with current reagents that used a colorimetric readout in as fast as 30 minutes for SARS-CoV-2 detection. Lau *et al.* (67) designed and optimized a sensitive reverse transcription recombinase polymerase amplification assay (RT-RPA) for the fast detection of SARS-CoV-2 utilizing SYBR Green I and/or lateral flow (LF) strips. In experimental systems, four classes of Clustered regularly interspaced short palindromic repeats (CRISPR)-derived genome editing agents are currently available: nucleases, base editors, transposases/recombinases, and prime editors (68). Cas12a (CRISPR-associated protein 12a) or Cas13a (CRISPR-associated protein 13a) nucleases are used in the most innovative forms of these researches that take advantage of collateral cleavage of single-stranded DNA (Cas12a) or RNA (Cas13a) (63). Broughton *et al.* (69) used the Cas12a method for COVID-19 diagnosis. The assay was created to detect regions in the SARS-CoV-2 E and N genes, and the human RNase P gene as a control (63). the SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) COVID-19 detection methodology based on CRISPR-Cas13 screens for unique nucleic acid targets (SARS-CoV-2 ORF1ab and S genes) and employs a dipstick as a visual readout in less than an hour. Other molecular methods, like microarray assays and viral sequencing (next-generation sequencing) can be utilized for the detection of SARS-CoV-2, however, their application is still restricted (58) (**figure 2**).

PATHOLOGICAL FEATURES

The main target organ of COVID-19 is the lung. The pathological features of COVID-19 are comparable to those of Middle Eastern respiratory syndrome (MERS) coronavirus infection and SARS (70). The lungs are heavy and congested with bilateral interstitial edema. Grossly visible pulmonary emboli and a distinctive patchy gross appearance of the lung parenchyma have been reported (71).

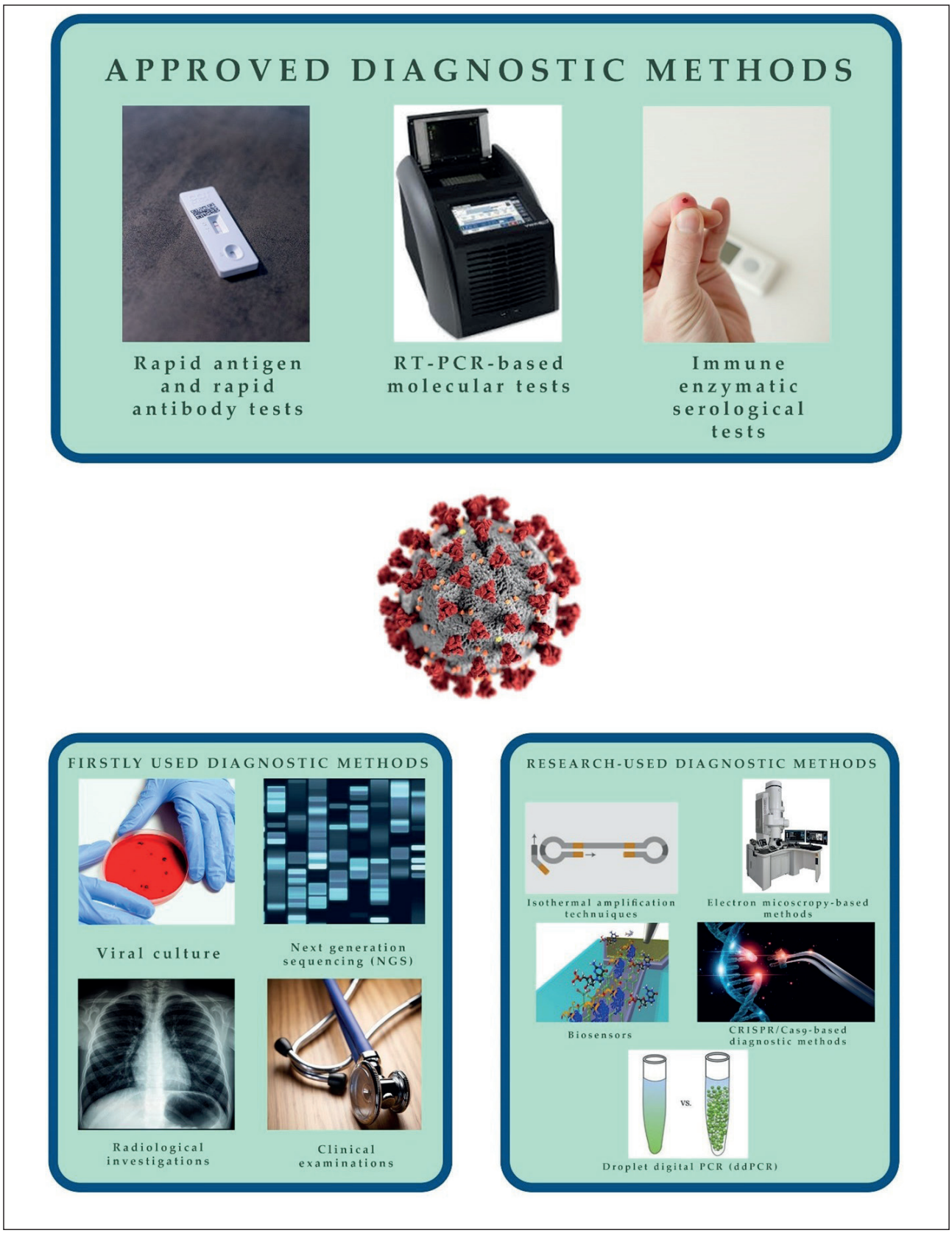


Figure 2. *Overview of the available clinical, diagnostic and research strategies for the effective diagnosis of COVID19 infection.*

Elsoukkary *et al.* (72) showed that the average total lung weight was 1,851 g (reference range = 685-1,050 g) in a postmortem study on 32 patients with COVID-19. Exudative and proliferative diffuse alveolar damage (DAD) were present in most of the patients (n = 24, 75%). The acute stage of DAD, as in other conditions, is marked by the presence of hyaline membranes, while the organ-

izing phase is characterized by variable degrees of the proliferation of fibroblasts and myofibroblasts (73). Pronounced fibroblastic proliferation, partial fibrosis, pneumocyte hyperplasia leading to interstitial thickening and collapsed alveoli, and patchy lymphocyte infiltration were the predominant findings in 10 confirmed cases of COVID-19 in the organizing-stage diffuse alveolar damage according to Schaller *et al.* (74). In areas with organized diffuse alveolar damage, reactive osseous and squamous metaplasia were seen (74). Xiao *et al.* (75) performed gastrointestinal endoscopy for a confirmed COVID-19 patient. Histological examination revealed damage to the mucosa in esophagus, and infiltration of numerous plasma cells and lymphocytes in the lamina propria of the stomach, duodenum, and rectum. The cytoplasm of gastric, duodenum, and rectal glandular epithelial cells were marked with viral nucleocapsid protein, but not in the esophageal epithelium, since ACE2 is rarely expressed in the esophageal epithelium. Lagana *et al.* (76) examined the liver sections of 40 COVID-19 autopsies. Grossly, two livers showed fibrosis and one had abscesses, the remaining livers showed varying degrees of steatosis, congestion, and ischemia. The most common histological findings were, macrovesicular steatosis, mild acute hepatitis, and minimal-to-mild portal inflammation. Santoriello *et al.* (77) showed that, acute tubular injury (ATI) was the most notable renal histologic finding in a group of 42 autopsied patients dying with COVID-19. The degree of ATI was most commonly mild, and ischemia, hypoxia, sepsis-associated factors, and toxin exposure could be suggested as the etiologic factors. Giavedoni *et al.* (78) showed that COVID-19 related cutaneous lesions could be classified into six patterns: Generalized maculopapular (20.7%), Grover's disease and other papulovesicular eruptions (13.8%), livedo Reticularis (6.9%), Other eruptions (22.4%), Urticarial (6.9%), and CHilblain-like (29.3%). In acral chilblain-like lesions, a diffuse heavy lymphoid infiltrate of the dermis, and the hypodermis, with a predominant perivascular pattern are seen (79). Fox *et al.* (80) detected the major gross and microscopic findings of 22 hearts from COVID-19 infection confirmed deaths. The hearts weighed 340-1010 gm. The most significant finding was severe right ventricular dilatation. Marked diffuse single myocyte necrosis was seen on microscopic examination. The endothelial cells of the small arterioles, venules, and capillaries were plump, and immunostaining showed diffuse perivascular infiltration of CD4 and CD8 lymphocytes. Lymphocytopenia, neutrophilia, eosinopenia, mild thrombocytopenia, and less frequently, thrombocytosis (81) are the most frequent hematological findings. Lymphocytopenia appears to be the most important change in the peripheral blood, and it can be used as a marker of severity of the infection (82). Harris *et al.* (83) investigated the bone marrow of 19 autopsied cases. They were all normocellular to hypercellular, with a myeloid shift, and hemophagocytic histiocytes were detected. Xu *et al.* (84) performed postmortem needle biopsies from the spleen on 10 patients who died from COVID-19 in Wuhan. The histopathological examination showed decreased cellularity of the spleen with atrophic white pulps at various ranges. The lymphoid follicles were diminished or nonexistent at all, and the ratio of red pulp to white pulp was variably increased. Liu *et al.* (85) noticed that the 12 postmortem spleens were all contracted and had shrinking capsules. The contracted spleens showed, mixed thrombi, anemic infarction, and hemorrhagic areas. Bryce *et al.* (86) studied the microscopic findings of the thoracic lymph nodes of 60 cases. Sinus histiocytosis was detected in 50 cases, 34 of which showed foci of hemophagocytosis. Germinal centers were lacking in 52 of the 60 lymph nodes. In 142 autopsies, gross brain findings were reported. The most remarkable abnormality was hemorrhage ranging from petechial bleedings to punctate subarachnoid hemorrhages (n = 9), and to massive cerebral or cerebellar hemorrhage (87). SARS-COVID-2 viral particles were found in the frontal lobe of the brain and endothelial cells of the capillaries. Perivascular acute disseminated encephalomyelitis (ADEM)-like picture, and neocortical microscopic infarcts were also observed in autopsy findings (88). Iuga *et al.* (89) revealed adrenal gland findings who described small vessels with acute fibrinoid necrosis, subendothelial vacuolization, and apoptotic bodies. Furthermore, Yang *et al.* (90) studied the 12 post-mortem testicular biopsies, and observed Sertoli cell swelling and detachment from tubular basement membrane, reduced Leydig cells, mild lymphocytic inflammation, and intratubular cellular sloughing. They reported immunohistochemical positivity to markers such as CD3, CD20, CD68, CD138, and ACE-2 as well (91). In another study, the olfactory bulbs were edematous and oval, and microscopic examination showed diffuse edema, inflammatory cell infiltration, severe neuronal degeneration, and

neuronal necrosis. Microglial nodules and scattered degenerative neurons were also observed in the ganglion cell regions (32).

TREATMENT

The most important symptomatic treatment for COVID-19 patients is oxygen therapy (92). The classes of drugs being evaluated or developed for the management of COVID-19 include antivirals, antibodies, anti-inflammatory agents, targeted immunomodulatory therapies, anticoagulants, and antifibrotics (28). The list of drugs for instance, in Tongji Hospital, Wuhan for the treatment of COVID-19 patients was as follows: Interferon-alpha (IFN-α), Lopinavir/ritonavir (LPV/r), Ribavirin, Chloroquine or hydroxychloroquine, Arbidol (93). Long *et al.* (93) showed that starting oxygen treatment less than 2 days following onset of hypoxic symptoms and the using of IFN-alpha among critically ill patients were both linked to a lower risk of COVID-19 mortality. Oral antivirals do not have the side effects of monoclonal antibodies, which must be administered in a hospital setting, and they are far less expensive (94). Remdesivir is a direct-acting antiviral drug that inhibits RNA-dependent RNA polymerase(RdRP) (95). It is an FDA-approved intravenous drug for use in adult and pediatric patients both older and less than 12 years of age for the treatment of COVID-19 requiring hospitalization (96). A 3-day regimen of remdesivir showed a tolerable safety profile among nonhospitalized patients at high risk for Covid-19 progression, and resulted in an 87 percent lower chance of hospitalization or mortality than placebo (97). The US Food and Drug Administration (FDA) issued an emergency use authorisation for Pfizer's COVID-19 antiviral, Paxlovid, on Dec 22, 2021. Paxlovid is a combination of two drugs: ritonavir plus the novel protease inhibitor PF-07321332. Paxlovid inhibits a protease that is needed for replication (98). On December 23, the FDA approved Merck Sharp & Dohme's (MSD) molnupiravir, an oral antiviral. Molnupiravir causes the replicating virus to accumulate mistakes until it can no longer survive (99). Monoclonal antibodies (mAbs) have emerged as valuable tools for treating and detecting a variety of diseases due to their high specificity and reliability. The receptor-binding domain (RBD) of the SARS-CoV-2 spike protein has become a primary target for therapeutic Ab development since it is critical for viral infection (100). Bamlanivimab is a strong neutralizing mAb (IgG1 with an unmodified Fc region) to the S protein that was generated from the convalescent plasma of a patient who had COVID-19 (100). The use of inhaled adenosine in COVID19 patients has resulted in a 6-day reduction in duration of stay. The modifying and regulating activities of adenosine on macrophages could explain its effectiveness (101). Purinergic receptors are important in understanding the COVID-19. P2X7 is one of the receptors recently discussed in COVID-19. It is ionotropic and has an affinity for ATP. P2X7R has been identified as a possible treatment target for COVID-19102 (102). Multiple drugs acting on different signaling pathways such as angiotensin-II receptor antagonists, blockers of RAS pathway, ACE inhibitors, inhibitors of serine protease such as TMPRSS2, and Tocilizumab, and Baricitinib inhibitors of the JAK/STAT pathway have been studied (103). Researchers are interested in the development of JAK inhibitors as a therapeutic intervention in COVID-19. The JAK/STAT pathway is involved in the release of cytokines and chemokines which regulate inflammation in organisms (104). The small molecular inhibitors are known to prevent the interaction of SP with ACE2 and other proteases. Arbidol, a membrane fusion inhibitor authorized for the influenza virus is currently being tested against COVID-19 in clinical trials (105). FDA has approved the emergency use of baricitinib (an oral JAK1/JAK2 inhibitor) (106), in combination with remdesivir, for the treatment of certain hospitalized patients with suspected or laboratory-confirmed COVID-19 (107). Compared with those who received standard treatment alone, 129 patients hospitalized for COVID-19, received tocilizumab (an IL-6 receptor-targeted antibody), in addition to standard treatment, were significantly less likely to need ventilation or die within 2 weeks (92). IL-6 is a major signal transducer and activator of transcription 3 (STAT3) stimulator, particularly during inflammation, and Hojyo *et al.* (108) hypothesize that IL-6-STAT3 signaling is a promising therapeutic target for the cytokine storm in COVID-19. Thromboembolic prophylaxis with subcutaneous low molecular weight heparin is recommended for all hospitalized patients with COVID-19 (28). Studies show that, the use of dexamethasone is associated with a lower risk of invasive mechanical ventilation and, for those already receiving invasive mechanical ventilation, a greater likelihood of early cessation (109). The hospitalized patients in New

York with COVID-19 who were treated with hydroxychloroquine, azithromycin, or both, did not have significant differences in in-hospital mortality compared to those with neither treatment (110). To decrease the virus-ACE2 connection, researchers are using the CRISPR-Cas9 technique to generate point mutations in human ACE2 (111) (**table I**).

Drug	Mechanism of action
Arbidol	Targets S protein/ACE2 interaction Inhibits membrane fusion of the viral envelope
Camostat mesylate	Inhibits TMPRSS2 Prevent viral cell entry
Tocilizumab **Sarilumab**	Bind IL-6 receptor Prevent IL-6 receptor activation Inhibit IL-6 signaling
Chloroquine **Hydroxychloroquine**	Inhibit viral entry and endocytosis by multiple mechanisms as well as host immunomodulatory effects
Lopinavir **Darunavir**	Inhibit 3-chymotrypsin-like protease
Ribavirin **Remdesivir** **Favipiravir**	Inhibit viral RNA-dependent RNA polymerase (RdRp)

Table I. *SARS-CoV-2 Potential Drug Targets* (112).

COVID-19 VACCINES

In the development of vaccines for viral diseases antigens are delivered to induce virus-specific neutralizing antibodies. Additional immunological responses may be needed for effective vaccine-induced immunity for many viruses, including CD4 and CD8 T cells with specific characteristics and positioning (113). Traditional immunization against viral infections is based on the use of the entire pathogen in a weakened or inactivated condition by chemical or physical alterations. To create immunological memory to a particular vaccine antigen, or even a toxin, inactivated vaccines which are replication-deficient or killed viruses or bacteria are administered (114). However, they typically give less protection for a shorter time and induce modest immunological responses, particularly cell-mediated immunity. As a consequence, inactivated vaccinations are given with a powerful adjuvant and require boosters to produce satisfactory and long-lasting immunity (115). Another method for vaccine development is to isolate viral proteins like the spike rather than the entire virus. The immune system reaction to the isolated protein is generally not as strong as it is to the full virus particle, but it is safer and easier to produce. Protein subunit vaccines have a low immunogenicity, and to produce a more robust immune response, an adjuvant must be included in the vaccine formulation (116). In SARS-CoV-2, the T cell response against the S, M, and N subunit proteins was found to be the most prominent and long-lasting (117). Injecting patients with RNA or DNA encoding viral proteins is a more advanced method of vaccine development (118). DNA vaccines are extremely stable and require no refrigeration, making them ideal for use in endemic areas (119). mRNA vaccines are an attractive alternative to traditional vaccine technologies because of their high potency, ability to generate quickly, and potential for low-cost manufacturing and safe delivery. However, owing to the instability and inefficiency of mRNA distribution in vivo, their usage was limited until recently (120). Adenoviruses (Ad) are one of the most widely used vectors for vaccine production, with Ad5 being the most frequently employed non-replicating Ad vector (121). The S protein or RBD subunit of SARS-CoV-2 is expressed in most of the vaccines based on non-replicating Ad5 viral vectors S (117). Because of its safety and lack of pre-existing immunity in humans, the chimp adenovirus (ChAdOx1) is a viable alternative to the human Ad vector (122). Virus-like particles (VLPs) have the same structure as viruses but lack the viral genome and are therefore non-infectious (123). S protein spikes on the exterior of the produced SARS-CoV-2 VLPs make them excellent for vaccine development (124). Unlike subunit vaccines, VLPs are unable to connect directly to B cell receptors to produce (117). Low productivity and high costs limit the use of cell-based vaccinations. For example, a "synthetic mini-gene" producing the SARS-CoV-2 viral proteins S, M, E, N, and polyprotein protease (P) was constructed using a lentiviral vector (LV-SMENP) and transmitted to artificial APCs (APCs) (NCT04276896) (117). Currently, more than 200 COVID-19 vaccine candidates are being developed using a variety of technologies. The two front-runner vaccines based on mRNA platforms, Pfizer/BioNTech BNT162b2 and Moderna mRNA-1273, have been approved by the US Food and Drug FDA for emergency use in mid-December 2020, with reported overall efficacy rates of 95 percent and 94.1

percent, respectively (125).

COVID19 AND ANIMALS

Based on the biological features of bats and the high identity sequence between bat-nCoV and SARS-CoV-2, bats are considered as the natural reservoir of SARS-CoV-2 for now. The intermediate host is thought to be the pangolin. Snakes, minks, and turtles, as well as ferrets and domestic animals, should not be overlooked (126). Hamsters are susceptible to SARS-CoV infection with comparable viral replication in the upper and lower respiratory tract (127). Rosenke *et al.* (128) found that the SARS-CoV-2 RBD has a strong functional interaction with the hamster ACE2 receptor. The pathological features of SARS-CoV-2 infected hamsters' lungs are like those seen in COVID-19 patients. Syrian hamsters are an excellent small animal model for testing vaccinations, immunotherapies, and antiviral medications (129). Mathavarajah and Dellaire (130) used the recently reported crystal structure of ACE2 and the RBD of the SARS-CoV-2 spike protein to try to figure out why dogs are less susceptible to SARS-CoV-2 than cats. They discovered that a mutation at amino acid H34 found solely in dogs (H34Y) and not in feline ACE2 was the fundamental distinction between these domestic pets. As a result, H34 appears to be a crucial residue linked to the species' susceptibility to the SARS-CoV-2 virus. H34Y is thought to reduce ACE2 and SARS-CoV-2 binding affinity. Strong ACE2 expression is found in tracheal and bronchial goblet cells, tracheobronchial submucosal gland serous epithelial cells, and type I and type II pneumocytes in cats. Cats can contract SARS-CoV-2 from their owners and are susceptible to experimental infection. They shed virus in the nasal turbinates, soft palates, tonsils, tracheas, lungs, and small intestines, with the live virus in all these tissues except the intestines and feces, implying minimal virus shedding via that route. There is currently no evidence of *cat-to-human transmission (131).* Other SARS-CoV-2 infections in pigs, dogs, chickens, and tree shrews have shown limited findings, with none demonstrating illness symptoms and only dogs shedding in feces but not tissue. Chickens have a high level of resistance to SARS-CoV, MERS-CoV, and SARS-CoV-2. In ferrets, the pattern of ACE2 expression resembles that in cats, except that it is absent in type I pneumocytes and tracheal and bronchial goblet cells (132). The ferret is a great small animal model that can mimic many of the manifestations of human influenza virus infection (133). Ferrets are a COVID-19 infection and transmission animal model that could aid in the development of SARS-CoV-2 therapeutics and vaccines (134).

COVID-19 AND CANCER

The pathogenesis of COVID-19 and cancer share certain similarities, with both expressing inflammation, immunological dysregulation, and coagulopathy (135). Hematological cancer, lung cancer, and breast cancer patients have more vulnerability toward getting infected with Sars-CoV2 (136). Inflammation is linked to the development of cancer and promotes carcinogenesis (137). Angiotensin-converting enzyme 2 plays an important role in the development of cancer (138). The expression of ACE2 is higher in some cancers such as lung, cervical, pancreatic, and renal carcinomas, while the expression is decreased in breast, prostate, and liver cancers. Patients, particularly with prostate cancer, have higher expression of TMPRSS2 as compared to patients with renal, lung, colorectal, or pancreatic cancers, while other cancers have no significant expression of TMPRSS2 (139). COVID-19 cancer patients had much higher mortality and severe disease than the general population, according to data from the COVID-19 and Cancer Consortium (CCC19) cohort study, which comprised 1,018 patients (92). A year after the COVID-19 outbreak and the initial lockdown, it is apparent that the disease has taken a high toll on cancer patients, affecting every stage from screening to diagnosis, and treatment (140). Around 40000 fewer people than normal started cancer treatment in the UK last year, and US hospitals have been deluged by COVID-19 cases, rendering patients with cancer unable to obtain timely care. WHO has reported that one in three European countries had partially or completely interrupted cancer care services early in the pandemic (141). The COVID-19 diagnostic delay in the UK is expected to result in a 9.6% rise in breast cancer deaths, 16.6% increase in colon-rectal cancer deaths, 5.3 percent increase in lung cancer deaths, and 6.0 percent increase in esophageal cancer deaths during the following 5 years (142). A survey of 155 countries by WHO found that 42% of countries had disruption of services for cancer prevention and treatment; the degree of disruption

was proportional to the extent of the pandemic in that country. The possible cause of this high risk to cancer patients with COVID-19 is surely the immunocompromised state of the patient (143). Coronavirus pneumonia brought about a 24% mortality in individuals with cancer while a 3% mortality was observed with noncancer patients (144). Most cytotoxic agents used in chemotherapy cause bone marrow suppression which could ultimately result in thrombocytopenia and neutropenia, this further makes cancer patients more susceptible to infections (144). Radiation therapy has also been reported to damage lymphocytes resulting in lymphopenia (145). In Northern Italy, the study conducted on 25 patients with cancer and COVID-19 showed a high mortality rate of about 36% compared to 16.13% in non-cancerous COVID-19 patients, and lung cancer was prominent among cancer patients (146). A study in a New York hospital system and a multicenter study in China demonstrated that patients with lung cancer had a higher risk of adverse outcomes when compared with other cancer types (147). According to Dai *et al.* (148), lung cancer was the most common cancer histology in infected patients (20.95%), followed by gastrointestinal cancer (12.38%), breast cancer (10.48%), thyroid cancer (10.48%), and hematologic cancer (8.57%) of 105 patients. The incidence of mortality in lung cancer patients infected with SARS-CoV-2 was reported to be up by four times (146). Although patients receiving chemotherapy appeared to be at a higher risk of severe illness from COVID-19, delaying chemotherapy is not advised, whereas individuals receiving only radiotherapy showed no significant differences in severe events when compared to individuals without cancer (92). The likelihood of radiotherapy being preferred by clinicians to other forms of cancer treatment is supported by the fact that in the UK, radiotherapy services decreased by only 10% during the 10-week lockdown from March to May 2020, compared with a 40% reduction in surgery (143). Data from both Italy and Latin America suggest that delivery of radiotherapy services was less affected than other modalities (143). Depending on the stage of illness, the progression of cancer can be a challenge for delaying procedures in cancer patients. Delays in the case of prostate, breast, cervical, or skin cancer in early stages can be tolerated but pancreatic, lung and hematological cancers such as leukemia require treatment as soon as possible (146). To avoid more suffering to patients from the deadly pair of COVID-19 and cancer, oncologists along with cancer societies, advise putting cytotoxic chemotherapy on hold and waiting until the SARS-CoV-2 virus becomes negative in the body (149). Cancer treatment regimens that do not affect outcomes of COVID-19 in cancer patients may not need to be altered (150). The presence of COVID-19 with malignancy makes diagnosis extremely challenging. Diagnosis of radiographs can be similar in both COVID-19 and cancer which may deceive the healthcare professional in making an accurate diagnosis (146). Carbohydrate antigens (CA) 125 and 153, carcinoembryonic antigens (CEA), human epididymis protein 4 (HE4), CRP, and cytokeratin-19 fragment (CYFRA21-1) are common markers in both COVID-19 and cancer; these markers are raised in both COVID-19 and cancer (146). Cancer patients are at a higher risk of both arterial and venous thromboembolism, especially if they are undergoing systemic chemotherapy (151). Cancer affects one's immune system and physiology through higher D-Dimer, lower levels of albumin, longer prothrombin time, and higher neutrophil counts (152). Sallah *et al.* (153) evaluated the occurrence of DIC in 1117 patients with solid tumors. Of these patients, 76 (6.8%) were diagnosed with DIC. Thrombocytopenia, hypofibrinogenemia, elevated D-dimer and, fibrinogen degradation products were the most common coagulation abnormalities encountered in patients with DIC. Tumoral factors such as tissue factor (TF), podoplanin, plasminogen activator factor (PAI-1), cytokines, NET, and mucins trigger the risk for thrombosis (146). The type of cancer changes with the severity of coagulation, *e.g.*, adenocarcinomas, lung cancer, pancreatic cancer, gastrointestinal cancer, and ovarian cancer have elevated risk for coagulation, while the risk is lower in breast and renal carcinoma compared to no risk associated with prostate cancer and melanoma (146). Cancer and COVID19 treatments generally have similar goals, and several anti-cancer medications are being examined in clinical trials to see whether they might be repurposed for COVID-19 (154). Recombinant IFNγ and IFNα2b have been widely utilized to treat cancer, and IFN administration has emerged as a promising treatment for COVID-19 (155). Cancer immunotherapy includes pharmaceuticals such as immune checkpoint inhibitors and monoclonal antibodies (MAbs), immunogene therapy, cell therapy, and vaccines (156). The same treatment regimen that is utilized to prevent or lessen cytokine storm in cancer patients receiving CAR-T cell therapy could be

used to lower the risk of cytokine storm in COVID-19 patients. As a result, antibodies that target the IL-6 receptor (tocilizumab and sarilumab), IL-6 (siltuximab), and other receptor antagonists (α1-adrenergic receptor antagonist, prazosin) for preventing cytokine storm are useful therapeutic options for the treatment of cancer patients with COVID-19 (157). PD1 inhibitors are immune checkpoint inhibitors (ICI), which have gained potential importance in solid cancer treatment (158). Reduction in sepsis or infection after pneumonia and inflammatory response syndrome was observed in COVID-19 patients administered with PD-1 inhibitors (146). ICIs would likely to be a protective factor against the onset of COVID-19 infection (159). Ruxolitinib a Janus-associated kinase (JAK) inhibitor has been reported to reduce cytokine-mediated inflammation, reducing severe events such as ARDS in COVID-19 infected patients, and many trials are currently active (146).

CONCLUSIONS

The results of COVID-19 imply that heavy polluters must act quickly and strongly on climate change to prevent a far more hazardous future and a more difficult recovery course (160). The COVID-19 dilemma teaches us a lot about what to expect in the forthcoming global climate crisis. Global emergencies are not new, but our ability to understand, avoid, and respond them has never been better (161). The only safe method to deal with COVID is to develop a vaccination, and the only way to combat climate change is to convert to a low-carbon system (162). Medications now being evaluated for COVID-19 repositioning can be divided into two categories: 1) medications that may impede one or more steps of the coronavirus lifecycle, and 2) drugs that may counteract the consequences of SARS-CoV-2 infection, such as the heightened immune response and massive cytokine release (163). More effective vaccines will need to be developed specifically for immunocompromised individuals. Several anti-cancer medications, in addition to antiviral and anti-inflammatory medications, could be repurposed to treat COVID-19 (154). SARS-CoV-2 can infect a wide range of animals. Several animal models, including the mouse, hamster, cat, ferret, and monkey, have been identified as suitable for evaluating the efficacy and safety of antiviral medicines or testing experimental vaccinations against SARS-CoV-2 (164).

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Authors' contribution

Conceptualization: AAN, FC, Dd, LC, FN and LA; writing-original preparation: AAN, FC, Dd, LC and FN; writing-reviewing and editing: AAN, FC, Dd, LC, FN and LA; supervision: FC and LA.

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NARRATIVE REVIEW

DIGITAL THERAPEUTICS IN ONCOLOGY: FINDINGS, BARRIERS AND PROSPECTS. A NARRATIVE REVIEW

G. Gussoni[1], E. Ravot[2], M. Zecchina[2], G. Recchia[3,4], E. Santoro[5], R. Ascione[2], F. Perrone[6]

[1] Research Department, FADOI Foundation, Milan, Italy
[2] Healthware Group, Milan and Salerno, Italy
[3] Smith Kline Foundation, Verona, Italy
[4] daVi DigitalMedicine, Verona, Italy
[5] Laboratory of Medical Informatics, Mario Negri Institute for Pharmacological Research IRCCS, Milan, Italy
[6] Clinical Trials Unit, IRCCS National Cancer Institute Fondazione Pascale, Naples, Italy

CORRESPONDING AUTHOR:

Gualberto Gussoni
Research Department, FADOI Foundation
Piazza Cadorna 15
20183 Milan, Italy
E-mail gualberto.gussoni@gmail.com
ORCID: 0000-0003-3573-5069

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ABSTRACT

Digital therapeutics (DTx) have been defined as technologies that "offer therapeutic interventions driven by high-quality software programs, based on scientific evidence obtained through methodologically rigorous confirmatory clinical investigation, to prevent, manage and treat a broad spectrum of physical, mental and behavioural conditions". DTx products are on the market in a number of countries or under development for a broad range of physical and behavioral conditions, including oncology treatment management. The aim of this narrative review is to provide an update on findings available for DTx, specifically developed for the treatment of patients with cancer.
A search was conducted using the following databases: PubMed, Google Scholar, Clinicaltrials.gov and Deutsches Register Klinischer Studien, as well as some websites specifically concerned with DTx. The products included in this review had to rely on at least one randomized controlled trial (already published or ongoing); or to be in the active phase of development for oncological indications, as documented by registered ongoing clinical trials and declared by the developer ("candidate DTx").
A total of nine DTx have been selected for this review, eight of them validated by Regulatory Authorities. The mechanism of action of DTx in oncological indications is mainly linked to cognitive behavioral stress management or management of symptoms and adverse events from anti-cancer treatments. In the majority of cases, quality of life, control of fatigue and physical activity/performance status were the primary endpoints of the studies. Survival was assessed in 3 studies, showing significant benefit in cancer patients using DTx.

Data available in the literature seem to indicate the prospect of a useful role for DTx in addressing many unmet needs that characterize the current management of cancer patients. The success of this path is linked to a series of significant aspects: need for more clinical research and evidence of clinical benefit on relevant outcomes; greater improved familiarity of physicians with these technologies, regulatory systems ready to evaluate the products, possibly also for reimbursement; and access to technology, together with improved digital literacy, for patients and caregivers.

KEY WORDS

Digital therapeutics; oncology; clinical validation; behavior; patient-reported outcomes.

IMPACT STATEMENT

Digital therapeutics are evidence-based devices aimed at interacting with the patient, and offer potential benefits for patients with cancer (reduced symptom distress, improved medication adherence, adverse event management, quality of life and survival).

INTRODUCTION

Digital therapeutics (DTx) have been defined as technologies that "*offer therapeutic interventions driven by high-quality software programs, based on scientific evidence obtained through methodologically rigorous confirmatory clinical investigation, to prevent, manage and treat a broad spectrum of physical, mental and behavioural conditions*" (1, 2). The key aspects to note in this definition of DTx are: (i) high-quality software programs; (ii) confirmatory evidence-based investigation; and (iii) therapeutic interventions. These distinguish DTx from other digital health products such as digital wellness apps, the various forms of software or hardware used to obtain measurements that could be useful for health purposes, or the so-called digital pills (*i.e.* pharmaceuticals with an integrated sensor that is activated once the drug arrives in the digestive tract, triggering a signal to an app housed on a smartphone in order to indicate that the treatment has been taken as prescribed).

Recognizing that DTx must be "confirmatory trial evidence-based" is crucial. Since the purpose of DTx is to obtain a clinically relevant effect, and a likely scenario for their place in therapy is that of a medical prescription, it seems reasonable to require that their prescription for a certain therapeutic indication is based on an experimental clinical validation comparable to that of other therapeutic products (*e.g.* a drug) prescribable for the same indication. In this perspective, various institutions have been working toward developing a standard for determining how much evidence is required to go to market or what type of evidence and regulation is needed (3).

While drug treatment interacts with the patient's biology, the main mechanism by which DTx achieve the therapeutic effect is through interaction with patients' thoughts, and correction of dysfunctional behaviors. In this perspective, another peculiar characteristic of DTx emerges, namely the active and participative involvement of the patient and/or caregiver, which is crucial to the success of the treatment pathway. As part of this pathway, DTx can work in a standalone modality, or in association / combination with drugs and other active treatment measures for the target pathology/clinical condition (4).

For illustrative purposes, we can propose an analogy between DTx and drugs. Looked at in these terms, a DTx product, which can take such different forms as an app (on a smartphone or tablet), a video game or a virtual reality system, may comprise an active principle and one or more excipients. Whereas in classical pharmacology the active principle is a chemical or biological molecule, in DTx it is the algorithm that constitutes the active element responsible for the clinical effect, whether positive (clinical benefit) or negative (undesired effects).

With regard to the discovery of the active principle, we have at least two main options:

- use a treatment already available in the scientific literature (*e.g.*, a tried and tested cognitive behavioral therapy), affording an alternative to administration of a known treatment;
- use a newly developed active principle, for instance, by setting up an original combination of different treatment modalities (*e.g.*, cognitive behavioral therapy, motivational interviewing, psychoeducation, *etc.*), based on the experience of the patient, the caregiver, the

medical specialist and the team of developers working on the algorithm.

As is the case with traditional drugs, the aim of the excipient is to "give shape" to the active principle and enable the patient to take it, making it as bioavailable – or, in this case, digitally bioavailable – as possible; for this purpose, reward and/or gamification modules introduce an element of patient gratification or of gaming into the dynamics of user interaction with the system. There may also be reminders to the patients that they must take the DTx product and complementary therapies, as well as modules to put them in touch with the physician and with other patients following the same therapeutic indication. The excipients can also include the user interface, which plays a fundamental role in making the therapy acceptable, ensuring patient compliance and, as a result, securing the expected therapeutic outcome (5). In addition, just like conventional drugs, DTx also have a well-defined indication and posology, which clearly differentiate them from digital health apps targeting consumers (not patients).

Today, DTx products are on the market or under development for a broad range of physical and behavioral conditions (mostly chronic) such as diabetes, anxiety disorder, depression, insomnia, attention-deficit/hyperactivity disorder (ADHD), substance use disorder, hypertension, chronic obstructive pulmonary disease, and oncology treatment management (6).

To have a quantitative idea of the research focused on these products and examine its geographical distribution, we explored the ClinicalTrials.gov register. By searching the strings "digital therapeutic OR digital therapy" in the "Intervention/treatment" field (January 17, 2022), we found 360 ongoing clinical studies in the area of DTx. As documented by a recent systematic review (7), however, it is important to note that these figures could be significantly overestimated as a result of misclassification, since product characteristics and study aims in many cases are not consistent with the above-mentioned definition of "digital therapeutics". **Figure 1** shows the geographical distribution of these ongoing studies, which are mainly based in North America (57%), followed by Europe (31%) and China (6%).

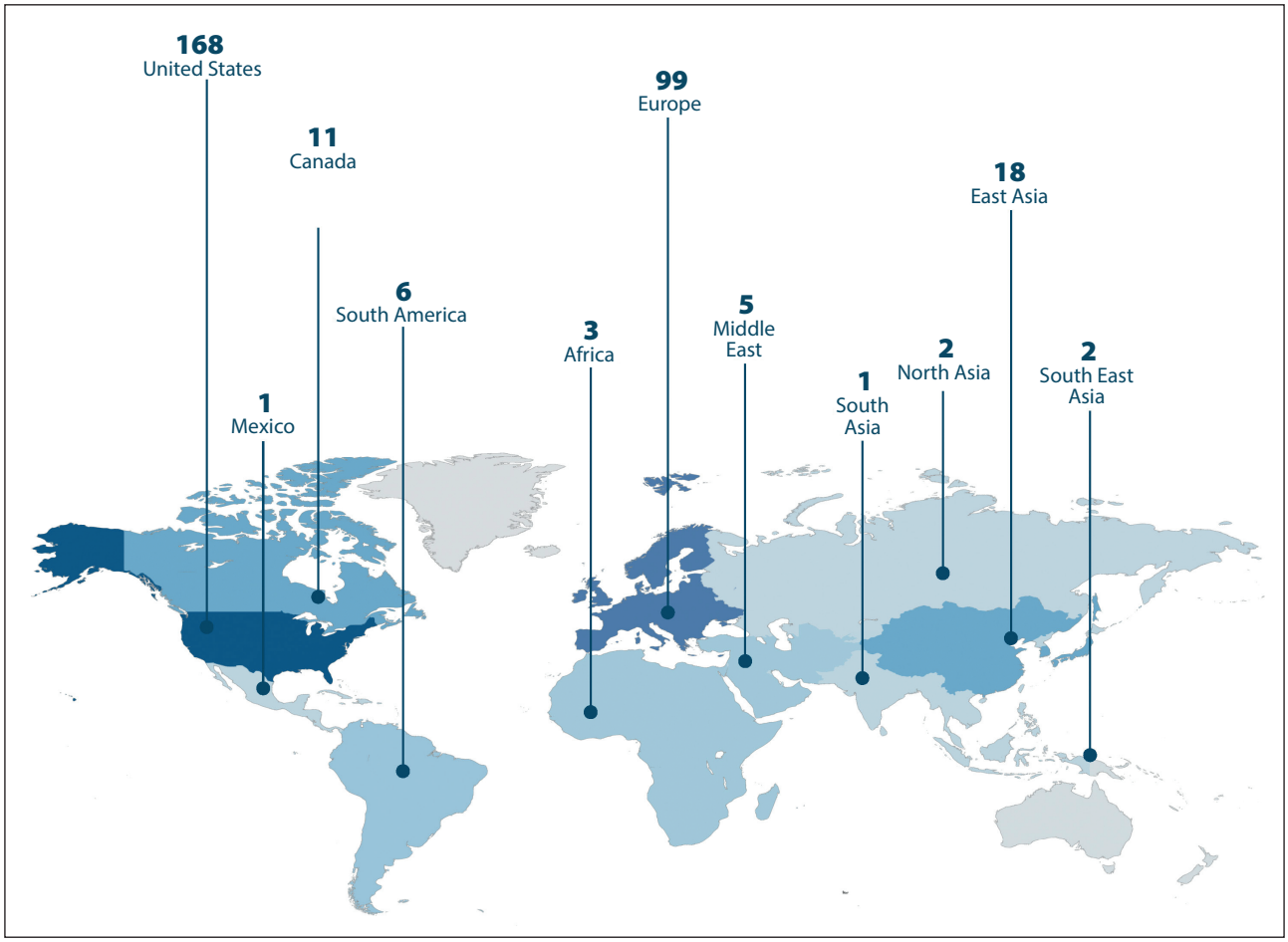

Figure 1. *Overview of geographical distribution of ongoing clinical trials in digital therapeutics* (source ClinicalTrials.gov).*

*Studies with no locations are not included in the counts on the map.

Studies with multiple locations are included in each region containing locations.

CANCER AND DTx

A global surveillance report suggests a trend toward increased survival for patients with cancer (8), with some tumors becoming increasingly chronic. The recent intensive development of therapies with novel mechanisms of action, including molecular targeted therapies, immuno-oncology therapies, and precision radiation oncology, has transformed the treatment landscape for cancer (9-11). These advances have increased the complexity of treatment (*e.g.*, combination of therapies) and required modifications in the patient pathway to ensure quality care. More drugs are available in oral formulations for home administration, with reduced face-to-face surveillance by healthcare professionals, and a greater likelihood of non-adherence and administration errors by patients (12); further, the prolonged use of such treatments as long-term maintenance may be associated with the emergence of toxicities (13). Additionally, a potential shortage in oncology services and workforce, linked to increasing cancer incidence and complexity of cancer treatments (14) has highlighted the need for new strategies to ensure that all patients receive optimal treatment and care throughout the *continuum* of disease, while also enhancing their ability to manage symptoms and treatment-related side effects. The new approaches should focus on patient-centred care, with integration of tumor-directed treatment alongside patient-directed supportive and palliative care throughout the disease journey (15, 16) (**figure 2**). The goals of management are to achieve improvements not only in overall survival (OS) but also in patient-reported outcomes (PROs), such as quality of life (QoL) (17), fewer emergency department visits, and self-reported improvements of symptoms (17, 18). Finally, since the total burden of new cancer cases is increasing, and new therapies are generally more expensive (19), novel approaches for optimal patient management allowing containment of healthcare costs are needed (20).

All these aspects underpin a strong rationale for an increasingly integrated approach to the management of cancer patients, embracing contributions from health products of various kinds, such as digital solutions, and with the aim of supporting both physical and mental health.

Digital health technologies for people with cancer include mobile apps for pain relief (21), self-management (22, 23), or videogames designed to promote physical exercise and mental empowerment in pediatric oncology patients (24, 25). Personalized digital interventions for oncology patients, with scope for provision of diverse self-care modalities such as physical exercises, yoga, mindfulness meditations and breathing exercises, can support anti-cancer therapies. Potential benefits of these interventions also include reduced symptom distress, decreased unplanned hospitalizations, as well as improved medication adherence, adverse event

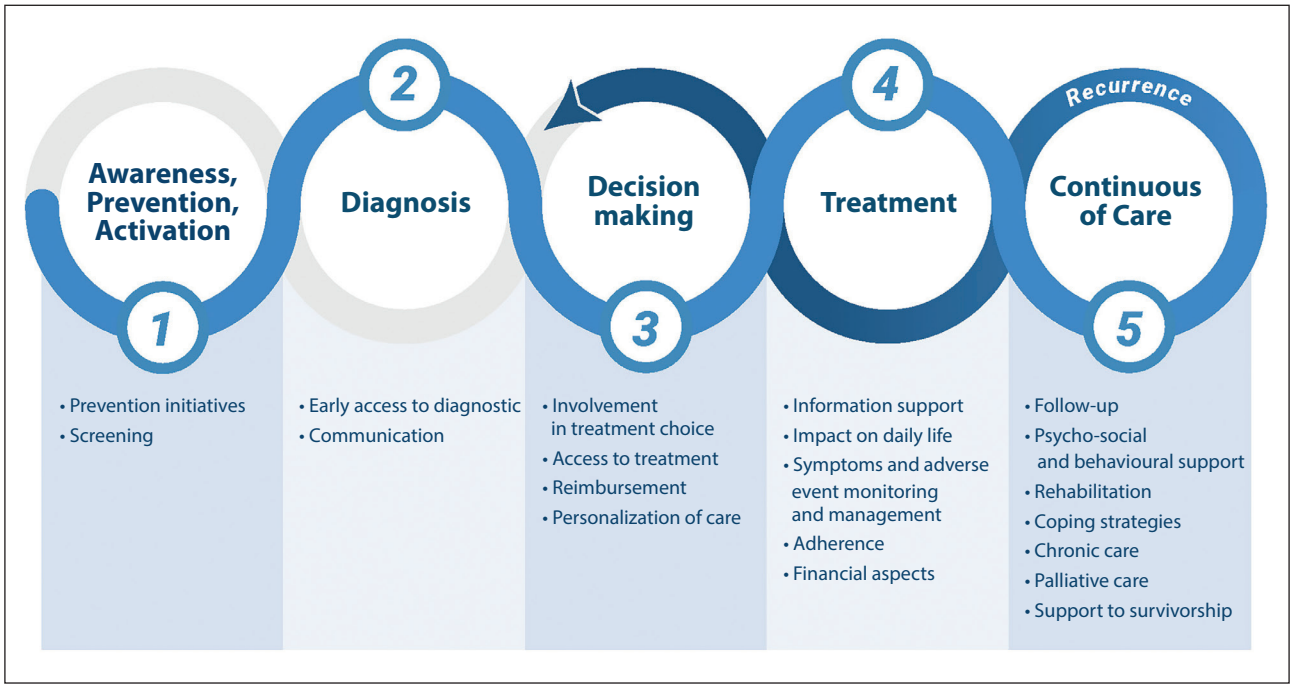

Figure 2. *Illustrative example of Patient Journey in oncology, with relevant touchpoints* (15, 16).

management, quality of life and survival (11). Among patients receiving treatment for advanced cancers, symptoms are common and frequently cause distress, functional impairment, emergency room visits and hospitalizations (26). Yet cancer patients' symptoms often go undetected and unaddressed by clinicians (27-29). Digital therapeutics are evidence-based medical devices, in which the active principle is represented by an algorithm/software (Software as a Medical Device – SaMD): this interacts with the patient by providing behavioral indications, collecting real-time data and clinical information which can be shared with the healthcare personnel and, if needed, favor timely therapeutic interventions. There is growing interest in integrating healthcare solutions such as DTx into routine practice for the management of chronic diseases, and the aim of this narrative review is to provide an update on the findings available for digital products classifiable as DTx on the basis of the above definition, and specifically developed for the treatment of patients with cancer. In many countries across the world, numerous other available DTx can be prescribed to cancer patients for the management of accompanying clinical conditions (*e.g.* depression, insomnia, abuse of opioids etc.): these products have not been considered for the purposes of this review.

METHODS

The MEDLINE Public Library of Medicine (PubMed) and Google Scholar databases were explored in the period September 2021-January 2022, for relevant studies published in the previous five years, and using the following search terms: (i) MEDLINE: digital therapies AND cancer; digital ther* AND (cancer OR oncology OR tumor OR neoplasm OR carcinoma); digital therapeutics AND leukemia; digital therapeutics AND lymphoma; (ii) Google Scholar (allintitle): digital therapeutics; digital therapeutics cancer; digital therapeutics tumor; digital therapeutics cancer approved; digital therapy cancer. Focused or selective searches were also performed on: (i) databases of clinical studies, in particular Clinicaltrials.gov and Deutsches Register Klinischer Studien (DRKS); (ii) websites or reports with a specific focus on DTx, in particular: BfArM/DiGa directory, Digital Therapeutics Alliance, daVinci Digital Therapeutics, Digital Therapeutics in the Oncology Market, and company websites of individual DTx.
Randomized controlled trials, observational studies, feasibility or pilot studies, editorials and reviews that evaluated efficacy/safety or effectiveness of DTx products in cancer patients were eligible for inclusion. Descriptions of the design of ongoing or planned studies available through the clinical studies databases or other sources were also considered. Search results were critically analysed by the authors for relevance to the focus of this review (**figure 3**).
The products included in this review and classified as DTx had to rely on at least one randomized controlled trial with confirmatory characteristics (already published or ongoing) ("DTx"); or to be in active phase of development for oncological in-

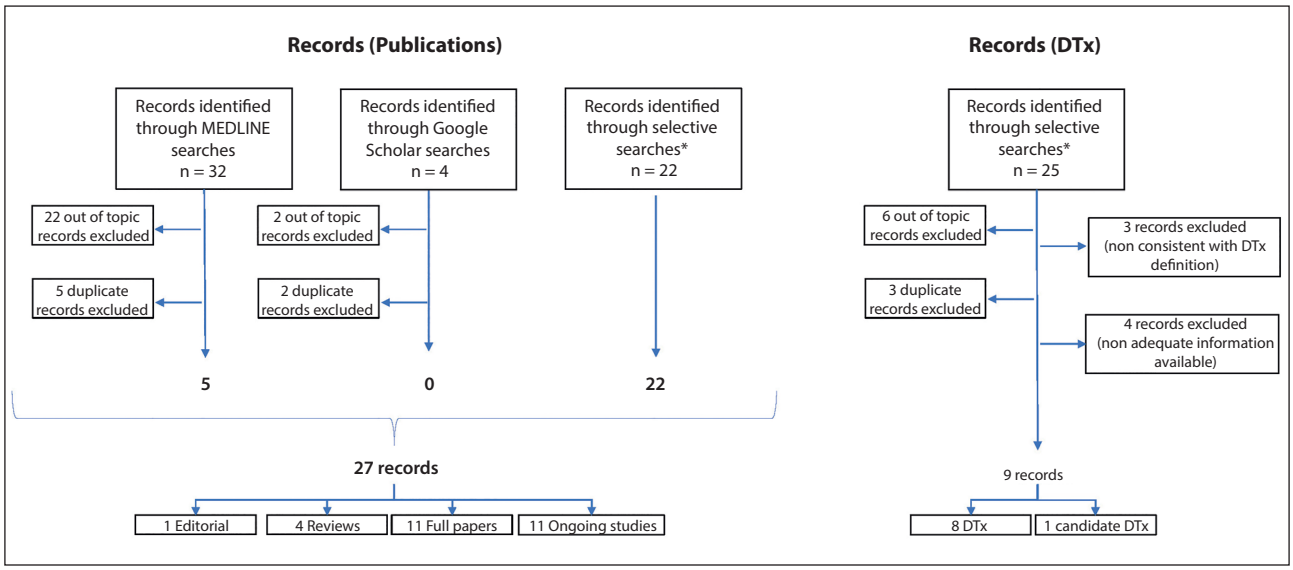

Figure 3. *Flowchart of methods and related results.*
*Clinicaltrials.gov (https://clinicaltrials.gov/), DRKS, BfArM/DiGa directory, ASCO Congress, Digital Therapeutics Alliance, daVinci Digital Therapeutics, Digital Therapeutics in Oncology Market and websites of Companies developing DTx.

dications, as documented by registered ongoing clinical trials and declared by the developer ("candidate DTx") (30, 31).

RESULTS

The main characteristics of 11 full papers reporting the results obtained with a total of 6 DTx in patients with cancer are summarized in **table I** (17, 18, 32-40). This list of papers includes controlled clinical trials (17, 18, 32, 33, 35-37, 40) or other types of studies, if they refer to products for which at least one randomized clinical trial was available in the literature, as additional findings contributing to their profile (34, 38, 39). Some papers (17, 18, 32, 33) refer to more than one product since they were based on the application of a web-based platform for symptom monitoring that was used for the development of digital tools by different companies. Generally speaking, the mechanism of action of DTx in oncological indications is mainly linked to cognitive behavioral stress management or management of symptoms and possible adverse events of anti-cancer treatments. In a couple of these studies, products' feasibility of use and ability to engage the patients were specifically addressed; in one of them, the attitude of healthcare professionals towards DTx, the integration of this technology into the clinical workflow and the opportunity for saving time by decreasing phone consultations and visits were indirectly evaluated (interviews/questionnaires). In the majority of cases, QoL was the primary endpoint of the study, together with dietary habits and physical activity/performance status. The study that enrolled the highest number of patients was specifically aimed at improvements in fatigue severity and reduction of fatigue interference with daily activities. Survival was assessed in 3 studies, showing significant benefit in cancer patients using DTx. Further, in one study patients receiving the digital intervention were able to tolerate continuation of chemotherapy longer than when receiving usual care (mean 8.2 *vs* 6.3 months, p = 0.002) (17). Finally, preliminary findings indicate a potential benefit of DTx in terms of healthcare organization and costs, by decreasing the need for phone consultations and visits.

Our search identified the design of a number of planned or ongoing studies concerning DTx or candidate DTx in oncology. **Table II** summarises 5 studies registered in the ClinicalTrials.gov database, 4 studies registered in the German clinical trial database (DRKS-BfArM/DiGA directory), and 2 presented at the 2020 ASCO (American Society of Clinical Oncology) Congress. This list includes both intervention and observational studies aimed at evaluating health status (complaints/symptoms, cancer-related distress, adverse events, QoL, disease progression and survival) as well as identification of promoters and barriers to implementation of DTx in clinical practice (41-51).

Figure 4 provides an overview of worldwide regulatory status for 8 DTx products selected according to the previously described criteria and specifically developed for cancer, with their indications and main areas of intervention. Further details on this topic are given in **table III**.

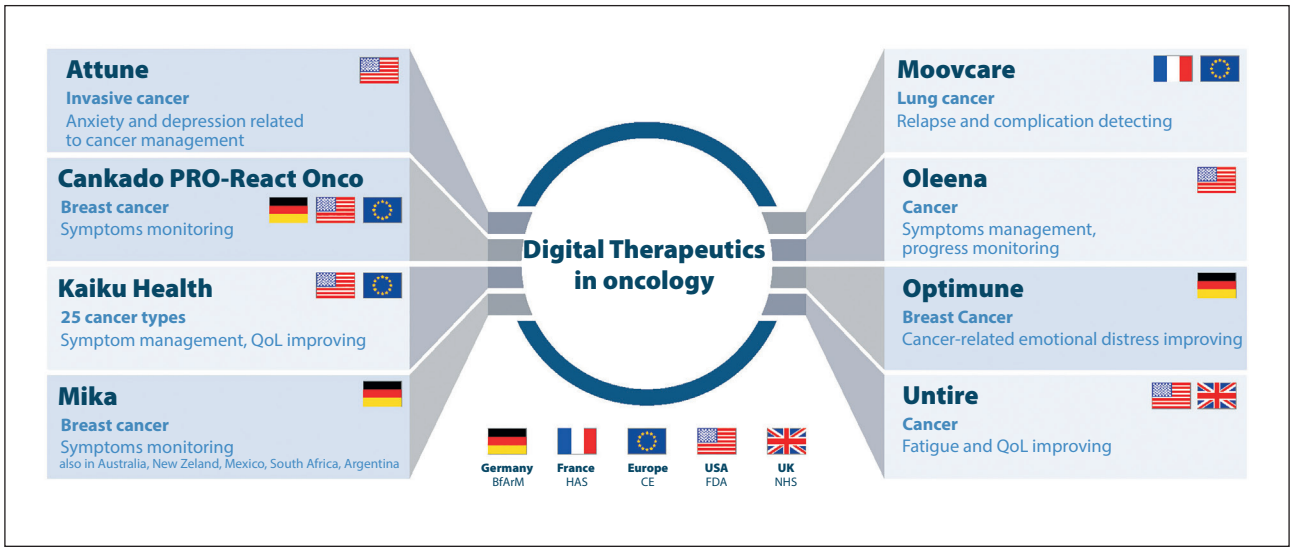

Figure 4. *Overview of DTx products with oncological indications.*

DTx	PAPER	PATIENTS	STUDY DESIGN	RESULTS
Moovcare Kaiku Health Oleena	Basch E *et al*. J Clin Oncol 2016 (18) Basch E *et al*. JAMA 2017 (17)	766 patients during chemotherapy for advanced solid cancer.	RCT–DTx for symptom monitoring *vs* conventional symptom monitoring. Assessment at 6 months. Analysis of survival.	HRQL (measured by the EuroQoL EQ-5D index) improved in 34% of patients of DTx group *vs* 18% among controls (p < 0.001). Significantly lower Emergency Room admission for patients in the DTx group (34% *vs* 41%; p = 0.02). Patients of the DTx group remained on chemotherapy longer (mean 8.2 *vs* 6.3 months; p = 0.002). Median OS = 31.2 months for DTx group *vs* 26.0 months for control (difference 5 months; p = 0.03).
Moovcare Kaiku Health Oleena	Denis F *et al*. Am J Clin Oncol 2017 (32)	98 patients with lung cancer.	Prospective follow-up using DTx *vs* conventional follow-up in historical group (retrospective). Median follow-up 12.3 months in the experimental arm *vs* 16.7 months in the control group.	Median OS = 22.4 months for DTx *vs* 16.7 months for control arm (p = 0.0014). One-year survival: 86.6 for DTx *vs* 59,1% for controls.
Moovcare Kaiku Health Oleena	Denis F *et al*. JAMA 2019 (33)	121 patients with lung cancer.	RCT – DTx *vs* routine follow-up. Two-year follow-up.	Median OS = 22.5 months for DTx *vs* 14.9 months for control arm (p = 0.03). Performance Status at the first detected relapse was 0-1 for 75.9% of patients in the DTx group *vs* 32.5% of controls (p < 0.001).
Oleena	Liu JF *et al*. JCO Clin Cancer Inform 2018 (34)	16 patients with ovarian cancer.	Pilot study to test feasibility, usability and perceived satisfaction to assist in managing acute treatment-related events (hypertension and diarrhea).	98,2% of expected BP values were reported; 87% of diarrhea events limited to grade 1. Hypertension and diarrhea events reported allowed rapid provider response and a positive overall patient experience.
Attune	Taub CJ *et al*. Cancer 2019 (35)	123 patients with stage 0-IIIb breast cancer.	RCT – 3 arms (CBT/RT/ Control-HE). 5-week experimental treatment. Assessment at 12 months.	Greater increases in stress management skills (MOCS) in combined CBT/RT groups *vs* HE (p < 0.001).
Attune	Penedo FJ *et al*. Int J Behav Med 2020 (36)	192 patients with advanced prostate cancer.	RCT – 10-week tablet-delivered CBSM *vs* HP. Assessment at 12 months.	Changes in HRQOL and symptom burden did not differ significantly between the groups. Men in the CBSM group reported greater improvement in self-reported ability to relax, both groups showed improvements in cancer-related anxiety and cancer-related distress
Untire	Spahrkas SS *et al*. Psychooncology 2020 (37)	799 patients with cancer-related fatigue.	RCT 2:1 ratio DTx *vs* control group. Assessment at 12 weeks.	The DTx group showed greater improvements in fatigue severity, fatigue interference and overall QoL on average (p < 0.01).
Kaiku Health	Iivanainen S *et al*. JMIR Form Res 2020 (38)	37 patients with advanced cancer treated with anti-PD-L(1).	Prospective, one arm study. Assessment at 6 months or until disease progression.	Electronic patient-reported outcome follow-up of cancer patients receiving ICIs is feasible and capture a wide range of symptoms.
Kaiku Health	Schmalz O *et al*. J Med Internet Res 2020 (39)	48 respondents (19 nurses, 8 physicians, 21 patients with advanced NSCLC treated with CIT).	Single arm intervention study Assessment 2 months (interim) and > 3 months after use of the DTx.	Most respondents agreed that the tool facilitated more efficient and focused discussions between patients and HCPs. The tool was well integrated into HCP daily clinical workflow, enabled workflow optimization between physicians and nurses, and saved time by decreasing phone consultations and patients visits.
Optimune	Holtdirk F *et al*. PloS ONE 2021 (40)	363 patients with breast cancer.	RCT – Usual care + DTx *vs* usual care. Assessment at 3 months.	The DTx group obtained significantly better effects on QoL (WHOQOL-BREF) and dietary habits (FQQ), and a non significant effect on physical exercise (IPAQ).

Table I. *Studies published as full paper and evaluating DTx products developed for cancer patients.*

BP: blood pressure; CIT: cancer immunotherapy; CBSM: cognitive-behavioral stress management; CBT: cognitive behavioral therapy; FQQ: food quality questionnaire; HE: health education; HER2: human epidermal growth factor receptor 2-negative; HP: health promotion; HR+: hormone receptors +; HRQoL: health-related quality of life; ICI: immune checkpoint inhibitor; IPAQ: international physical activity questionnaire; MOCS: measure of current status; NSCLC: non-small cell lung cancer; OS: overall survival; PD-L(1): programmed cell death protein 1- ligand 1; QoL: quality of life; RCT: randomized controlled trial; RT: relaxation training; SAE: serious adverse events.

DTx	DATABASE IDENTIFIER/ REFERENCE AND STATUS	PATIENTS	STUDY DESIGN	OBJECTIVES
ApricityRx	NCT04571398 (41) (Recruiting)	100 patients with cancer, receiving treatment with immune-checkpoint inhibitor.	Observational, prospective cohort study. Assessment at 12 weeks.	Evaluate DTx mobile app to capture and transmit to care team patient-generated health data and access education content on immune-related adverse events and immuno-oncology therapy.
ApricityRX	Campbell MT *et al*. J Clin Oncol 2020 (42)	1000 patients with cancer, is receiving treatment with immune-checkpoint inhibitor alone or in combination.	Single arm, open label study.	Evaluation of the effectiveness of ApricityRX as a mitigation strategy for immune-related adverse events.
Attune	NCT04862195 (43) (Recruiting)	553 patients with stage I-III breast cancer or stage I-III non small cell lung cancer.	RCT - to compare the effectiveness of two DTx (Attune and Cerena). Assessment at 10 and 12 weeks.	Primary endpoint: improvement in anxiety symptoms (week 10). Secondary endpoint: improvement in depressive symptoms (week 12).
Attune	NCT04857008 (44) (Recruiting)	20 patients with diagnosis or history of invasive cancer and mild to moderate anxiety/ depression.	Open label (single group), delivery of cognitive behavioral therapy. Assessment at 4 and 12 weeks.	Identification of promoters and barriers to clinical implementation. To measure changes in pre- and post-cancer-related distress (assessed with the VSAS / Veterans Symptoms Assessment Screen tool).
CANKADO-PRO React Onco	NCT04531995 (45) (Not-yet recruiting)	166.000 patients with cancer under systemic anti-tumor or anti-hormonal therapy.	Observational (patient registry) prospective cohort study. Assessment at 6 months.	Health status (EuroQol visual analogue scale). Complaints/symptoms. Presence or absence of: - serious adverse events - dose reductions - treatment interruptions - disease progression - disease regression - death
CANKADO PRO React Onco	https://diga.bfarm.de/de/verzeichnis/961 (46) (Recruiting)	Patients with breast cancer.	Intervention, comparative *vs* conventional management.	Evaluate the effects on general state of health (EQ-VAS) and patients' empowerment/health literacy.
CANKADO Pro React Onco	Degenhardt T *et al*. J Clin Oncol 2020 (47) (Recruiting)	Patients with HR+/ HER2- locally advanced or metastatic breast cancer.	RCT, *vs* inactive inform arm.	Evaluate the effects of the digital tool on the incidence of serious adverse events. Preliminary unplanned analysis on a secondary endpoint (primary study endpoint: QoL).
Mika	DRKS00026038 (48) (Recruiting)	250 adults patients with malignant tumor (diagnosis within the last 5 years).	RCT - 12 weeks Mika-App (+ TAU) plus 9 months for an additional longitudinal follow-up.	Primary endpoint: DT reduction (DT-VAS). Secondary endpoints: improvement QoL (CGI-I), fatigue reduction (FACIT-F), depression/anxiety reduction (HADS-D), adherence/compliance, patient sovereignty, health competence.
Mika	DRKS00021064 (49) (Recruiting)	70 adult patients with cancer receiving immunotherapy.	RCT - 12 & 24 weeks TAU + SOFIA / Mika App plus.	Primary endpoint: HRQoL (EORTC QLQ C30). Secondary endpoints: depression (PHQ9-D); generalized anxiety disorder (GAD7); distress (National Comprehensive Cancer Network/NCCN Distress-Barometer); supportive care needs (SCNS-SF34-D).
Mika	DRKS00022996 (50) (Recruiting)	524 patients with malignant tumors.	RCT, standard of care + Mika app *vs* standard of care (12 weeks).	Primary endpoint: quality of life. Secondary endpoints: psychological burden, psychological stress, fatigue, fear for progression, health literacy, adherence, self-management.
Moovcare	NCT04934865 (51) (Not-yet recruiting)	240 patients with lung cancer.	Intervention, single group study.	Evaluation of the proportion of patients whose management has been modified at least once and specially by Moovcare Lung application at 12 and 24 months.

Table II. *Selected clinical studies (planned or ongoing) with DTx products already authorized or under consideration for oncological indications, registered in the database ClinicalTrials.gov or in the German Register of Clinical Studies (accessed January 13, 2022), or presented at ASCO Congresses.*
DT: psychological distress; HRQoL: health-related QoL; QoL: quality of life; RCT: randomized controlled trial; SOFIA: e-Health application (managing Symptoms OF ImmunotherApy); TAU: therapy as usual.

DTx	AVAILABILITY
Attune	USA: class II medical device, only available by prescription. Please note that the treatment has not been reviewed by the U.S. FDA and is currently the subject of ongoing clinical trial evaluation.
CANKADO (Starter/PRO-React Onco)	EU: approved class I medical device. Germany: temporary approval for breast cancer according to DVG. USA: compliant with the FDA classification for Mobile Medical Devices (2015) Appendix B.
Kaiku Health	EU: CE-marked class IIa medical device. USA: device that falls under the FDA's enforcement discretion. Australia: class I medical device. New Zealand, Mexico and South Africa: registered as a medical device. Argentina: not regulated by medical device regulation.
Mika	Germany: transitory approval for cancer/ gynecological cancer (multiple cancer indications) according to DVG.
Moovcare	France: class I medical device / first digital therapy to be approved and reimbursed by the HAS. EU: authorized EU representative.
Oleena	USA: approved as medical device for prescription by FDA.
Optimune	Germany: free of charge as part of a scientific study.
Untire	UK: approved by the NHS and listed in the NHS App Library. UK, USA: ongoing negotiations with insurers and healthcare providers for reimbursement for cancer fatigue.

Table III. *International regulatory status for DTx products with oncological indications.*
DVG: Digitale-Versorgung-Gesetz; EU: European Union; FDA: Food and Drug Administration; HAS: Haute Autorité de Santé; NHS: National Health System; UK: United Kingdom.

DISCUSSION

Digital health technologies offer new opportunities to integrate health promotion, self-care and lifestyle interventions, while simultaneously mitigating limitations of pharmacotherapies such as tolerability, medication nonadherence, or drug resistance. In the specific field of oncology, digital solutions can address certain unmet needs related to better control of symptoms, as well as prevention or management of adverse events in patients with cancer, including: (i) increased communication between patients and healthcare professionals; (ii) education and empowerment of patients and caregivers; (iii) integration of standard clinical assessments with PROs measured during routine clinical practice; (iv) help for patients in monitoring and self-managing their conditions (52). All these opportunities can increase access to treatment, improve the safety and quality of care with better health outcomes, and decrease medical costs (11, 53). These benefits, in addition to being evaluated in trials, could be studied in the real world more easily than is the case with drugs, thanks to the opportunities that arise from the use of digital tools.

Among digital health technologies, DTx are evidence-based medical devices aimed at interacting with the patient by providing behavioral and therapeutic indications; there is growing interest for their integration into clinical practice, and this review is focused on products of this category specifically developed for cancer. In this perspective, a first non-trivial point is the risk of misclassification of a digital tool in the category of DTx, as highlighted through a recent systematic review by Santoro et al. (7). This may have influenced our bibliographic search, based on keywords that included the terms "digital therapeutics"/"digital therapies". According-

ly, our choice for this review was to consider as DTx those products for which at least one randomized controlled trial with confirmatory characteristics was available in the literature or ongoing; or those tools in the active phase of development, as documented by registered ongoing clinical trials and development plan ("candidate DTx"). These criteria excluded a number of digital tools for which information is available online (54-58). Further, we considered the different products' ability to deliver a "therapeutic intervention" (another key aspect for the definition of DTx). This too is a delicate point: for example, the dividing line between DTx and technologies classified as "digital support program" can be a very fine one, particularly in the case of cancer. In fact, the mechanism of action of DTx used in this indication mainly depends on the reaction of healthcare personnel to alerts related to toxicities and symptoms and reported by the patient. Being the digital tool a trigger of the intervention, this can therefore be considered a particular form of "therapeutics". However, for the products selected for this review, the authors considered that their classification as DTx was adequate, in relation both to their disease management purposes and to the presence of an experimental clinical validation program.
The mechanisms of action of DTx for cancer patients are generally related to cognitive behavioral stress management, teaching and empowering patients about the significance of key symptoms, vital signs and possibly drug-related adverse events, as well as the real-time reporting of health status and outcomes; and a digital interaction between patients and healthcare personnel that enables the latter to triage, evaluate and treat in a timely fashion. According to the results of available studies, this can lead to improved QoL, physical activity and performance status, as well as of reduced severity of specific symptoms such as fatigue which are particularly common among cancer patients and significantly interfere with their daily activities. These effects were reported in patients with various cancer types, the most frequently represented being breast, lung and prostate cancer. Of particular relevance is the report, documented in some randomized controlled trials, of a significant increase in survival among patients using these digital tools, with orders of magnitude difficult to achieve even through pharmacological therapies of proven efficacy. Once again, potential factors underlying this finding are integration of PROs into the routine care of patients and early responsiveness to patient symptoms, preventing adverse downstream consequences and, where possible, identifying relapses in a timely manner. Moreover, patients receiving the digital intervention were shown to tolerate continuation of chemotherapy longer than usual care (17), and this can contribute to increased survival. Finally, preliminary findings indicate a decreased need for consultations and visits in patients using DTx, thus suggesting a potential benefit in terms of healthcare organization and costs.
The adoption of DTx, both in general and in the specific field of cancer, is complex: it often involves an array of different priorities in various fields, numerous decision-making processes, and individual or organizational value judgments (59). In part, this is believed to be due to the obscurity of the path to market, obstacles in finance and reimbursement, variance in the practice of medicine, regulation and security, and the absence of a structured process surrounding the evaluation and authorization of digital health products (60). Clinician and personal privacy issues, equal access, clinical effectiveness, and safety concerns are also significant potential barriers facing the adoption of DTx (61). A crucial point in meeting these concerns is, first of all, the value of the clinical benefit that DTx can bring, starting from the evidence provided by clinical studies; in other words, how to guarantee adequate, uniform efficacy and safety standards, similar to those for drugs used in the same therapeutic indication. While recognizing that the risk of obsolescence necessitates rapid lead times for DTx development, that their peculiarities must be taken into account at the study design phase, and that different types of studies (*e.g.* observational, or interventional single arm) may be useful for definition of the product's profile, randomized controlled trials represent the ideal model for pivotal clinical investigation of DTx. These must be carried out on an adequately sized sample (particularly in confirmatory studies), so that significant effects can be statistically demonstrated and be satisfactory for clinicians, Regulatory Authorities and possible other payers. In this perspective, some limits still exist for DTx specifically developed for cancer indications, and this was reflected in our literature review. The number of randomized controlled trials is low, and that of studies with a pre-defined and statistically adequate sample size is even lower. Further, it is not clear in how many of these studies the definition of the design and the objectives benefited from the active participation of patients, an aspect that is probably even more critical for DTx than for traditional pharmacological therapies. In addition, publication

bias cannot be ruled out, since there are appreciably fewer published papers than there are studies registered in international databases (*e.g.* ClinicalTrials.gov). These are crucial issues that need to be addressed, in order to favor a more substantial and evidence-based place of DTx in therapy of oncological indications.

On the other hand, the data available in the literature seem to indicate the prospect of a useful role for digital tools in addressing the many unmet needs that characterize the current management of cancer patients. The promising findings on patient-reported outcomes/QoL, symptoms and overall survival lend weight to the view that a digitally supported and systematic engagement of patients can plausibly lead to better chronic control of the disease and improved outcomes. Our review was focused on the results obtained by digital products specifically developed for cancer indications, but we must not forget that in many countries a number of extensively studied and widely used digital tools (including DTx) are already available, with indications for clinical conditions frequent in cancer patients (*e.g.*, depression, insomnia, abuse of opioids).

Important progress towards a place in therapy for DTx in oncology is being achieved, but this has been the case only in the last few years. The road ahead is therefore still long, demanding and challenging, especially considering the enormous impact of cancer on the population and on public health resources. One of the major challenges is - and will continue to be - the effective application of virtual care models for cancer survivorship, in order to support patients living with the chronic effects of cancer treatment while also increasing health care capacity and sustainability (62). The ideal digital solution in the setting of supportive care in oncology would be user-friendly, intuitive, and engaging, so as to meet the immediate needs of the end-users. It would not be meant as a replacement for the practitioner, but as an efficient source of real-time complementary information, appropriate to the care of cancer patients and the specific related issues. Sufficiently detailed but not over-complicated, this information would be presented in language the patient understands, with a view to achieving effective symptom self-management (63). The digital solution would maintain existing expectations regarding patient confidentiality and data privacy (64), cybersecurity, compliance with regulatory requirements, and alignment with the most recent evidence-based practice. It would be operational throughout the entire course of the disease and for the range of different anti-cancer treatments, as well as sufficiently flexible for adaptation to different territories, settings and care needs (11).

It is likely that DTx will demonstrate their strengths in the current and future medical scenario, increasingly enabling the healthcare system to deliver personalized and customized medical care. However, apart from how well DTx are developed (and this is the most important point), their success and broad-based adoption is linked to overcoming a series of significant issues: need for strong evidence of clinical benefit on relevant outcomes/endpoints; difficulties in getting physicians to indicate and prescribe DTx due to lack of familiarity with these technologies; perceived difficulty in use; absence of standards and of a consolidated process for providing prescriptions; reimbursement arrangements; regulatory systems; and access to technology and improved digital literacy for patients and caregivers. As previously pointed out, clinical validation is a crucial point for DTx. The development of these products could benefit from a greater specific digital and methodological competence of researchers, including Health Technology Assessment. The latter, together with the recognition as an innovative product, is a critical dimension of the regulatory path also for the DTx, especially for healthcare contexts in which publicly funded assistance prevails, and the DTx aspire to reimbursement.

The awareness and engagement of patients is of paramount importance for the success of DTx, and in this perspective a fundamental role could be played by patient organizations. But one of the most important aspects that must be governed for an effective implementation of DTx in clinical practice concerns their management by healthcare personnel. Resources needed for the success of DTx are not only on the side of software, hardware and connection to the Internet, but also in terms of human resources and availability of time (*i.e.*, nurses and physicians who monitor collection of data and react to alerts). If this issue is not successfully addressed, and the DTx fail to be integrated into the normal care pathways, their ability to effectively intervene on the quality of life and clinical outcomes of patients, risks being significantly compromised.

This means that all stakeholders - governments, health systems, digital therapy entrepreneurs, pharmaceutical companies, payers, providers, researchers, physicians and patients - must agree on a common model. Only in this way will it prove

possible to define, develop, evaluate from the clinical evidence standpoint, commercialize, and distribute these products in line with adequate standards. This will make it possible to improve patient health and wellbeing, potentially decrease medical costs, and ensure widespread access to these products in a safe and effective manner. More clinical research, in terms of large-scale trials, and related systematic reviews and meta-analyses, could help significantly with a view to reaching these goals.

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ETHICS

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There were no institutional or private fundings for this article.

Conflict of interests

ER and MZ are employers at Healthware Group; GR is CEO at daVI DigitalMedicine srl which signed a joint partnership with Polifarma SpA for research and marketing of digital medical devices; FP received institutional grant for research activities from AstraZeneca, Bayer, BioClin, Incyte, Jannsen, Merck, Pfizer, Roche, Sanofi, Tesaro and honoraria for educational or advisory activity from Astellas, AstraZeneca, Bayer, Clovis, Incyte, Ipsen, Jannsen, Pierre Fabre, Roche, Sanofi; GG, ES and RA have declared no conflict of interests.

Availability of data and materials

No new data associated with this article.

Authors' contribution

Conception and design of the work: GG. Acquisition, analysis and interpretation of data for the work: GG, ER, MZ. Drafting the article: GG, ER, MZ. Revising the article critically for important intellectual content: GR, ES, RA, FP. Provide approval for publication of the content: all the authors.

Ethical approval

N/A.

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BRIEF REPORT

GONADOBLASTOMA: A BRIEF REPORT

R. Di Fiore[1,2], A. Agius[3], C. Camenzuli[4], S. Suleiman[1], J. Calleja-Agius[1], C. Savona-Ventura[5]

[1] Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta
[2] Sbarro Institute for Cancer Research and Molecular Medicine, Center for Biotechnology, College of Science and Technology, Temple University, Philadelphia, PA, USA
[3] Department of Psychiatry, Mount Carmel Hospital, ATD9033 Attard, Malta. andee.agius.03@um.edu.mt
[4] Department of Surgery, Faculty of Medicine and Surgery, University of Malta, Msida, Malta
[5] Department of Obstetrics & Gynaecology, Faculty of Medicine & Surgery, University of Malta, Msida, Malta

CORRESPONDING AUTHOR:

Jean Calleja-Agius
Department of Anatomy
Faculty of Medicine and Surgery
University of Malta
Birkirkara Bypass
MSD 2080 Msida, Malta
E-mail: jean.calleja-agius@um.edu.mt
ORCID: 0000-0001-6369-0297

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ABSTRACT

Gonadoblastoma typically occurs in dysgenetic gonads, but may rarely also involve a normal ovary or testis. Approximately 40% of such tumors are bilateral. Among affected individuals, up to 80% are phenotypic females and the rest are phenotypic males. Most patients with gonadoblastoma have 46,XY karyotype or various forms of mosaicism. The gonads are usually abnormal, with hypospadias, cryptorchidism and internal female secondary sex organs, which are either in the inguinal region or intra-abdominally. Gonadoblastomas are considered to be clinically benign neoplasms, but up to 50% are accompanied by foci of malignant germ cell tumor, mostly seminoma, and occasionally yolk sac tumor, embryonal carcinoma, choriocarcinoma or teratoma. This paper presents a brief literature review based on a case of an XY female reared as male, with development of a gonadoblastoma in the left ovary.

KEY WORDS

Gonadoblastoma; rare tumor; disorder of sex development; ovary; testis.

IMPACT STATEMENT

Gonadoblastomas are rare gonadal tumours, which are usually benign but they may sometimes become malignant if not treated. This paper presents a case report of gonadoblastoma in a case of intersex followed by a brief literature review.

INTRODUCTION

Gonadoblastoma is a rare tumor which consists of more than one type of cell (germ, stromal and granulosa cells) normally found in the gonads (ovaries and testes) (1). Although gonadoblastomas tend to be benign, they may occasionally turn malignant if left untreated. In up to 60% of cases, gonadoblastomas are associated with malignant germ cell tumours: typically, pure dysgerminoma or less frequently as yolk sac tumour, immature teratoma, embryonal carcinoma or choriocarcinoma (2). The majority of patients with gonadoblastoma present in infancy and young adulthood with abnormal gonads and have certain chromosome mutations. Here, we present a case of a gonadoblastoma in an individual with intersex, followed by a brief literature review.

CASE REPORT

At birth, a baby was noted to have ambiguous genitalia with no gonads palbable externally. Karyotyping was done and was reported as 46,XY. A diagnosis of severe hypospadias with undescended testis was made. In his teens, at the age of 16 years, he was investigated for marked gynaecomastia and hypogonadism. Hormone profiling revealed a follicle stimulating hormone (FSH) level of 99.4 U/L (males < 25 U/L; LH of 79.2 U/L (males < 25 U/L); and testosterone level of 1.5 nmol/L (males 12.5-34.3nmol/L). Being genotypically male, his parents consented that he could undergo a bilateral inguinal hernia repair, correction of hypospadias and bilateral subcutaneous mastectomies. The gonads were assumed to be intraabdominally placed. At the age of 19 years, he presented with abdominal pain with signs of peritonitis. On examination, he was found to be febrile and tachycardic at 128 bpm. He had an acute abdomen and blood investigations revealed a high white cell count (maximum 11.6 x 10^9 per cmm). A diagnosis of appendicitis was made and an open appendectomy was carried out via an incision over McBurney point. Histology confirmed a necrotic appendicitis. Additionally, a tube-like structure was identified and excised during surgery was reported to be a pyosalpinx with secondary peritonitis. At the age of 25 years, the patient presented as an emergency with anpther episode of acute abdominal pain and signs of peritonism (febrile with a pulse rate of 120bpm). Blood investigations revealed a high white cell count (maximum 19.2 x 10^9 per cmm). He gave a long history of having had monthly episodes of recurrent 'haematuria'. An intravenous pylogram had revealed no abnormality in the urinary tract.

A laparotomy was performed. At operation, a torted left-tubo-ovarian mass, with normal uterus and right ovary-looking gonad were found. Both gonads were removed at surgery. Histology revealed a gonadoblastoma in the left ovary and left pyosalpinx. The right ovary contained follicular cysts and degenerating corpus luteum. The patient was prescribed long-term testosterone supplementation.

In summary, this is a case of an intersexual disorder with incomplete virilization of the external genitalia requiring multiple corrections for what appeared to be hypospadias; and normal female internal genitalia which were functional as evident by monthly "haematuria" and surgical and histological findings. Karyotype was 46,XY, probably with absence of HY antigen – mullerian inhibiting factor.

BRIEF REVIEW ON GONADOBLASTOMA

The origin of gonadoblastoma and related entities

Gonadoblastoma was first described by Scully in 1953 (3), who then went on to report a case series

of 74 patients over a time frame of 17 years (4). Classical gonadoblastoma arises from undifferentiated gonadal tissue within the dysgenetic gonads of a person having a Y chromosome (or part thereof) and a disorder of sex development (5-9).

The precursor of classical gonadoblastoma has been proposed to be undifferentiated gonadal tissue in dysgenetic gonads, which has been identified in 67% of cases (5). The germ cells in both classical gonadoblastoma and undifferentiated gonadal tissue are heterogeneous and can express octamer-binding transcription factor 4 (OCT4), testis-specific protein, Y-linked 1 (TSPY1) or both (10). The expression of OCT4 in undifferentiated gonadal tissue can determine the delay in maturation of germ cells and, consequently, the risk of carcinogenesis in dysgenetic gonads (11). The last step in the transition to classical gonadoblastoma may be the clonal expansion of germ cells and final organization in undifferentiated gonadal tissue (12). The cellular gonadal stroma, which is typical of undifferentiated gonadal tissue, can sometimes form a small part of classical gonadoblastoma (13).

A different model has been proposed where classical gonadoblastoma was hypothesized as arising from 'dissecting gonadoblastoma' (14). The "dissecting" variant is believed to be a significant intermediate step in between the development of classical gonadoblastoma and germinoma. The latter is the likely precursor of other more malignant germ cell tumors including embryonal carcinoma, immature teratoma, yolk sac tumor and choriocarcinoma. Expression of SF1 or α-inhibin in the "dissecting" variant has been investigated to identify residual sex cord elements and differentiate it from germinoma (13).

There is far less known about those cases of gonadoblastoma that occur in females with a normal 46,XX karyotype or in males with a normal 46,XY karyotype and no evidence of a sex development disorder. Rarely, gonadoblastoma occurs in normal females with a 46,XX peripheral karyotype and no evidence of a disorder of sex development (15-19). Although the underlying cause is still unknown, it is very likely that these tumors arise through a completely different molecular pathway than the classical gonadoblastoma occurring in individuals with a disorder of sex development.

Clinical and histological features

Gonadoblastoma is a rare tumor that is more likely to occur in individuals with a sex development disorder, especially in phenotypic females (80% in phenotypic females versus 20% phenotypic males) (20). The commonest predisposing mutations are 46,XY complete gonadal dysgenesis; 46,XY disorder of sex development; and 45,XO/46,XY partial gonadal dysgenesis (5). Affected individuals usually have abnormal gonads with hypospadias, cryptorchidism, and internal female secondary sex organs located in the inguinal or intra-abdominal region. Although by definition, in individuals who have a disorder of sex development have at least one gonad which is developmentally abnormal; however, this abnormality may not be detected histologically if the gonad is completely replaced by the tumour. To date, only one case of gonadoblastoma has been reported in a 46,XY phenotypic female with androgen insufficiency syndrome and an associated germinoma (21).

Histologically, gonadoblastoma is a noninvasive neoplasm which consists of rounded islands or nests of cells surrounded by a variably cellular stroma. The rounded islands are composed of germ cells that are intimately mixed with immature sex cord derivatives, commonly surrounding hyaline basement membrane deposits or, rarely, calcifications. The germ cells present in individual cases of gonadoblastoma are heterogeneous, consisting of both mature and immature forms (6). Very often, gonadoblastoma undergoes involutional changes, leading to calcification and the formation of deposits of hyalinized basement membrane material. Occasionally, the involutional changes are extensive, resulting in a calcified mass without any viable neoplastic cells. This is referred to as involuted or "burnt out" gonadoblastoma (22, 4).

Classical gonadoblastoma contains 2 types of germ cells: the mature and immature. The germinoma-like cells have been shown to be the precursor of the malignant germ cells leading to gonadoblastoma. However, other germ cells resemble spermatogonia, but vary in nuclear size (23, 13). The mature germ cells express TSPY1, whereas the immature germ cells express OCT4. There is a small subpopulation of germ cells which coexpresses both proteins (10). The sex cord cells demonstrate cytoplasmic expression of α-inhibin and nuclear expression of steroidogenic factor 1 (SF1).

In individuals with a disorder of sex development, the differentiation of testis in dysgenetic gonads can be analysed using the transcription factors SRY-box 9 (SOX9), while ovarian differentiation can be visualized using forkhead box L2 (FOXL2) (24). The sex cord element of gonadoblastoma has been shown to express only FOXL2, and not SOX9.

Cases of ovarian mixed germ cell–sex cord stromal tumor can be mistaken for gonadoblastoma, especially in normal females. Criteria have been developed for distinguishing these from gonadoblastoma in females with a 46, XX peripheral karyotype and no evidence of a disorder of sex development (22). Ovarian mixed germ cell–sex cord stromal tumor characteristically has a diffuse growth pattern and lacks the numerous rounded islands of tumor nests surrounded by a basement membrane or the degenerative changes of hyalinization, which are typical of classical gonadoblastoma. Moreover, ovarian mixed germ cell–sex cord stromal tumor normally lacks basement membrane, basement membrane material, or calcifications (25). Furthermore, gonadoblastoma contains both benign and premalignant germ cells. In contrast, ovarian mixed germ cell–sex cord stromal tumor contains germ cells of only one type that is typically benign in those neoplasms occurring in the testis, and malignant in those tumors present in the ovary.

Management of gonadoblastoma

Upon diagnosis, individuals with gonadoblastoma undergo surgery, and in cases with malignant germ cell component, this can be followed by chemotherapy. Prognosis depends on the characteristics of the malignant germ cell component. However, excellent outcome has also been reported in cases of dysgerminoma (18, 26).

It is important to take into consideration the preferences of the individuals involved when planning the management of individuals with gonadoblastoma and a concomitant disorder of sex development. The ultimate functionality of the gonad is very relevant to this decision. In order to reduce the risk of sex dysphoria, patients' advocacy groups tend to be in favor of a more conservative approach whenever medically feasible, with the aim of avoiding possible gonadectomy, or at least delay it in those children till they are capable of giving their own informed consent (22). The individual described in this case had been identified to be chromosomally an XY individual and had his external genitalia altered to function as a male. When emergency surgery revealed the patient to have a gonadal tumour and an ovarian-looking gonad on the contralateral side, a decision was made to remove both gonads. Such a decision should ideally be carried out electively after a full chromosomal, anatomical, and psychological profiling is carried out to identify the true gender orientation of the individual. If an informed decision is made to remove the intra-abdominal gonads, then these can be removed through a laparoscopic approach, possibly also including the removal of existing Mullerian tube structures.

Following gonadotectomy, the patient was supplemented with long-term testosterone replacement therapy to prevent the symptoms of reproductive hormones withdrawal and to increase virilization features. Several testosterone supplement preparations are available these being administered orally (testosterone undecanoate 237 mg twice daily), transdermally (testosterone 40.5 mg per day), as subcutaneous pellets (crystallized testosterone every six months), or by intramuscular injection (testosterone enantate 250 mg every 3-6 weeks or testosterone undecanoate 1000 mg every 10-14 weeks). The choice of supplementation form is dependent of patient preference but the use of long-term supplementary testosterone should be monitored to screen for any significant increase in haematocrit that may place the individual at increased risk of thrombotic episodes. Individuals with a prostate should be monitored with regular prostate specific antigen (PSA) screening since the supplementation may increase the risk of prostatic cancer development (27).

CONCLUSIONS

Since the first case of gonadoblastoma was described, advances have been made in our understanding of the pathophysiology of this rare neoplasm. The majority of cases of gonadoblastoma occur in individuals with a disorder of sex development and an abnormal karyotype. However, some cases have been reported in normal individuals with no evidence of a disorder of sex development. Although there is a risk of occasional errors in diagnosis, criteria have now been established for distinguishing gonadoblastoma from ovarian mixed germ cell-sex cord stromal tumor in individuals who have a normal karyotype and no evidence of a disorder of sex development.

ETHICS

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Conflict of interests

The authors have declared no conflict of interests.

Availability of data and materials

All the data supporting the findings of this study are available within the article and can be shared upon request to the corresponding author.

Authors' contribution

Writing including the original draft, review, and editing was performed by RDF, AA, CC, SS. Writing including review and editing was performed by all the authors. This work was supervised by JC-A and CS-V. All authors have read and agreed to the published version of the manuscript.

Ethical approval

Ethics approval has been obtained from the Faculty Research Ethics Committee at the Faculty of Medicine and Surgery, University of Malta (FREC ID: MED-2022-18).

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BRIEF REPORT

ACCESS TO EARLY PHASE CLINICAL TRIALS AT THE TIME OF THE COVID-19 PANDEMIC: AN ITALIAN SURVEY

P. Lombardi[1], R. Falcone[1], M. Filetti[1], V. Altamura[1], R. Giusti[4], F. Paroni Sterbini[1], A. Pietragalla[2], S. Duranti[2], G. Scambia[2,3], G. Daniele[1,2]

[1] Phase 1 Unit, Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy
[2] Scientific Directorate, Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy
[3] Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy
[4] UOC Oncologia Medica, AOU Sant'Andrea, La Sapienza University of Rome, Italy

CORRESPONDING AUTHOR:

Gennaro Daniele
Phase 1 Unit
Scientific Directorate
Fondazione Policlinico Universitario A. Gemelli, IRCCS
largo A. Gemelli 8
00168 Rome, Italy
E-mail: gennaro.daniele@policlinicogemelli.it
ORCID: 0000-0001-5360-1895

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ABSTRACT

Italy was among the first countries hit by the pandemic of coronavirus disease 2019 (COVID-19). The application of strict lockdown measures disproportionately affected both cancer patient care as well as cancer research.

A survey was conducted among Italian oncologists to explore whether and how the COVID-19 outbreak has changed their aptitudes and practice toward early phase studies before and during the COVID-19 outbreak and suggestions to overcome the early phase clinical trial limitation in our country.

A total of 137 physicians completed the survey. In the pre-pandemic period, 103 responders (75.2%) declared a positive aptitude at referring their patients to early phase unit but only 12.6% referred more than 10% of their patients. Of these, 35% declared a reduction in this aptitude during the pandemic period. The majority of responders believe that the COVID-19 pandemic will affect the new oncological drug's marketing (62.3%). Over the COVID-19 pandemic, the majority of participants highlighted the necessity of an "alliance" between leader and satellite centers (59.8%), making the early phase unit distribution's homogeneous on the national territory (37.2%).

Our work provides an overview of the impact of the COVID-19 outbreak on aptitude at referral to early phase clinical studies among Italian oncologists.

KEY WORDS

COVID-19; survey; early phase studies; Italian oncologist; AIOM.

IMPACT STATEMENT

These data offer a snapshot of the aptitude at referral to early phase clinical studies during COVID-19 outbreak.

INTRODUCTION

Since the beginning of 2020, the coronavirus disease 2019 (COVID-19) pandemic has progressively affected millions of people worldwide and has disrupted many aspects of clinical care. According to the World Health Organization (WHO), as of October 20 2021, there were 241.411.380 confirmed cases of COVID-19, including 4.912.112 deaths (1).
Several evidences highlighted a higher risk of death from COVID-19 for cancer patients (2-6). During the first wave of the pandemic, all levels of care (screening, diagnosis, treatment, and follow-up) were disrupted. Moreover, cancer centres started prioritizing care services, cancelling non-urgent appointments, adapting treatment protocols, and shifting to home-based remote care relying on telemedicine consultations (4, 7).
Clinical research was affected by several aspects. Due to the difficulties generated by lockdown conditions, several trials have been interrupted or stopped with a substantial reduction of 74% of patients enrolled in clinical trials in May 2020 compared with the same period in 2019 (8). Concurrently, the reorientation of human and economic resources towards COVID-19 research has further affected clinical trial research. All of these aspects, the disruption and the fast, effective readjustment to address a new challenge, will lengthen the effects of the COVID-19 pandemic on clinical trials research for long after the initial effects have faded (9).
In a communitarian effort, many societies such as the European Society for Medical Oncology (ESMO) and the American Society of Clinical Oncology (ASCO) published recommendations to provide practical guidance for oncologists and patients. However, this crisis also highlighted the shortcoming of the clinical trial conduction and the need to optimize the bureaucratic system and the use of resources in clinical research (10-12).
Even before the COVID-19 pandemic, important barriers affected the conduct of clinical trials and the changes implemented due to the COVID pandemic were mandatory by the need to ensure the safety of patients, clinical researcher and staff (13, 14).
In this paper, we aim to photograph the aptitude to refer patients at early phase clinical studies among Italian oncologists, the impact of the COVID-19 pandemic on that aptitude and use them as a stimulus to launch a discussion over a framework of broader adaptations needed in the design and implementation of oncology clinical trials in our Country.

METHODS

A specific online questionnaire was sent by e-mail throw the institutional mailing list to all members of the Italian Association of Medical Oncology (AIOM) on May 11, 2021. The survey was open access and was also available to members through the AIOM website and social media channels. Participation was voluntary and anonymous. To deploy the questionnaire rapidly and for very fast data collection, a web-based modality was chosen. The Google Forms platform was chosen to implement the survey, and responses were automatically stored in a database built with Excel (Microsoft Office).
The survey was proposed to physicians involved in clinical oncological activities in both academic and clinical centres. Twenty questions were asked, including multiple-choice, closed- and open-ended questions and were divided into 4 different sections: 1) demographic, medical training and employment information (questions 1-8); 2) aptitudes and practice toward early phase studies before the COVID-19 outbreak (questions 9-11); 3) aptitudes and practice toward early phase studies during the COVID-19 outbreak (questions 12-17); 4) aptitudes and practice toward early phase trials and research activities regardless of the COVID-19 outbreak and suggestions to overcome limits highlighted (questions 18-20). The questionnaire was composed of 20 questions: 65% closed answers with single-choice selection (n = 13) and 35% open

questions with free-text response possibilities (n = 7) (**Supplementary survey**). Responses were described as frequencies and percentages. Statistical analyses were done with SPSS for Windows, version 27.0.

RESULTS

A total of 137 physicians completed the survey. Responses were collected between 11 May and 17 July, with 69% of responses registered in the first 72 hours. The majority were medical oncologists (124/137, 90.5%). Most of the respondents were female (74/137; 54%), aged between 30 and 45 years old (78/137; 57%) and worked in Northern Italy (64/137; 47%). The most common places of work were university hospitals (43/137, 31%) and specialised cancer centres (39/137, 28.5%) (**table I**).

Out of 137 responders, 49 (35.7%) worked in a dedicated Early Phase Unit Trial at the time of survey compilation. The disease of interest was lung cancer for 49 responders (35.8%) followed by gastrointestinal cancer (47/137, 34.3%) and breast cancer (43/137, 31.4%) (**supplementary figure 1**).

Clinicians were asked to compare their aptitude to refer patients at phase I clinical trial Units in the pre-pandemic and pandemic period. One hundred-three responders (75.2%) declared a positive aptitude to refer their patients to early phase Unite in the pre-pandemic period of which only 12.6% (13/103) referred more than 10% of their patients (**figure 1**).

CHARACTERISTICS OF RESPONDERS		
	N = 137	%
Gender		
Male	63	54
Female	74	46
Age		
≤ 30 years old	9	6.6
> 30 - ≤ 45 years old	78	56.9
< 45 years old	50	36.5
Italian geographical area of work		
Northen Italy	64	46.7
Central Italy	60	43.8
Southern Italy	23	9.5
Work setting		
University hospitals	43	31.4
Specialised cancer centres	39	28.5
General hospital	33	24.1
Private centre	9	6.5
Territorial medicine	13	9.5

Table I. *Demographic, Working regions, and Employment Information of the Responding Oncologists.*

This aptitude is widespread among all responders and no difference was observed among different subgroups. In fact, we observed a high aptitude of referral regardless of sex (male 74.6% and female 75.6%), age (≤ 45 years old 75.9% and > 45 years old 74%), geographic work area (78% north, 68% centre, 82.6% south) and place of work (university hospital 79%, specialised cancer centre 82%, general hospital 72.7%, private centre 77.7% and territorial medicine 46.15%). Among those who usually did not refer patients to an early phase clinical trial Unit (34/137, 24.8%), 44.1% (15/34) do not have a nearby centre or do not know how to contact the trial Unit and 38.2% (13/34) declare difficult contact with the early phase units that make it difficult to update which studies are open and with active enrolment.

Out of 103 participants that usually refer patients to the early phase Unit, 35% (36/103) declared a reduction in this aptitude during the pandemic period. Of these responders, 91.7% (33/36) correlate this reduction with the difficulties related to early phase trial conduction during pandemic (*i.e.*, difficult to reach the dedicated centre, the necessity of multiple visits to the hospital or to oncological centres that increase the infection risk and fewer trials available). Moreover, half of the physicians (15/36) declared a difficulty related to pandemic implication and change in professional duties (*i.e.*, increase in care load, involving COVID-19 clinical activities).

In the last section, we investigated implications for early phase and drug development after the COVID-19 outbreak and suggestions to overcome the limits of an early clinical trial in our Country. Out of 137 responders, thirty-eight (27.7%) believed that the COVID-19 pandemic will not affect the new oncological drug's marketing. The remaining parts believed that there will be a substantial delay due to the fewer patients enrolled and the delay of the early-phase trial (44/137, 32.1%) or fewer studies opened in this period (28/137, 20.4%) or for the use of technical and financial resources to face the pandemic (32/137, 23.4%). Interestingly, a sizable fraction of researchers believed that more than 12 months would be necessary to return to the pre-pandemic levels in terms of clinical research in oncology (55/137, 40.1%) (**supplementary table I**).

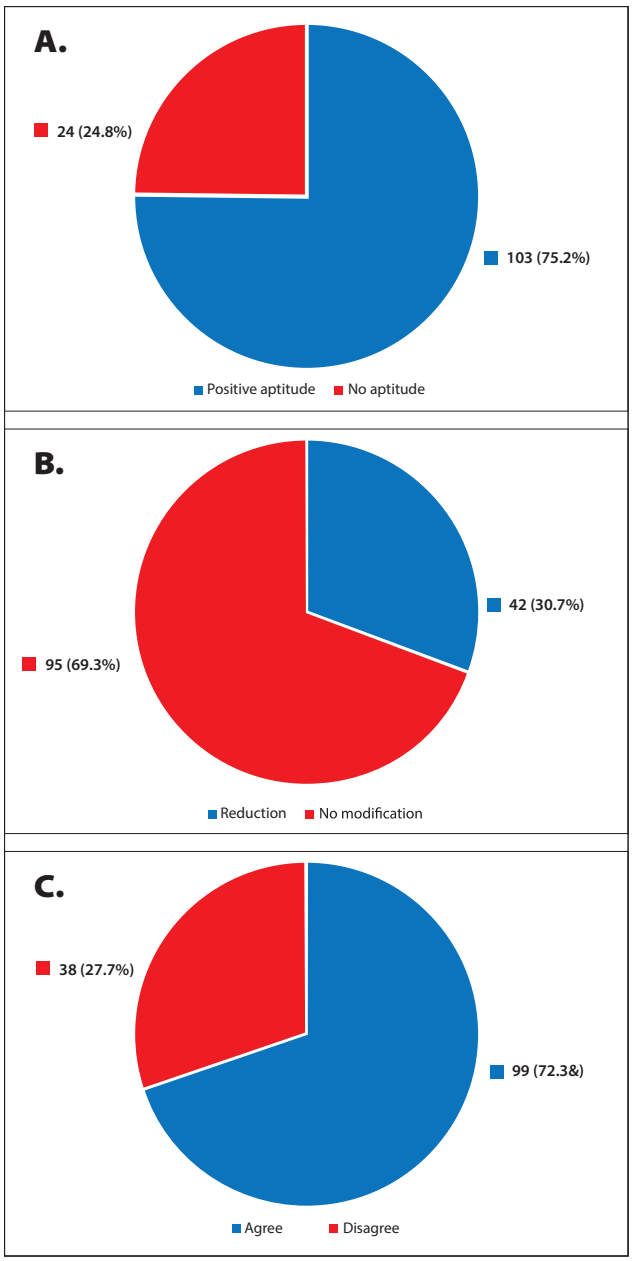

Figure 1. A. *pre-pandemic aptitude at referring patients to early phase clinical trials;* **B.** *modification in the physicians' aptitude at referring during pandemic;* **C.** *Concerned that COVID-19 will negatively affect drug-marketing authorization in the new years.*

The majority of participants highlighted the necessity of an "alliance" between leader and satellite centres (82/137, 59.8%), making the early phase Unit distribution's homogeneous on the national territory (51/137, 37.2%) and improving telemedicine to reduce visits (38/137, 27.7%) (**supplementary table II**).

DISCUSSION

This survey provides evidence on a diffuse, positive aptitude of Italian oncologists for referring patients' to early phase clinical trials. This tendency was distributed homogeneous among responders and it was independent of sex, age and working region or setting.

Nevertheless, we noted that, although this aptitude is rather diffuse among physicians, the percentage of patients usually referred to early phase clinical trial units is limited with only 12.6% of responders referring more than 10% of their patients. Numerous reasons could explicate these data, but one of the most important reasons is the asymmetric distribution of Phase I centres on national territories, and the ongoing phases I study concentrated in a few locations (15, 16). Furthermore, in a classification considering the phase I trials conducted worldwide between 1999 and 2020, Italy ranks only 14th position, following other similar European countries (*i.e.*, Germany, France, Spain, and the Netherlands) (17). The low availability of studies in our country and the logistical difficulties associated with an uneven distribution limit the number of patients that could enter an early phase trial. COVID-19 outbreak has disrupted all aspects of health care worldwide with a particular impact on oncological care and clinical trial (2-4). We did not know the long-term implications of this emergency situation but the majority of responders believe that the COVID-19 pandemic will negatively affect the new oncological drug's marketing with a large part of researchers believing that more than 12 months will be necessary to return to the pre-pandemic levels in terms of clinical research in oncology.

Nevertheless, only a minority of physicians declared a reduction in referring aptitude during the pandemic period. In our national context, we may speculate that this correlates with an "a priori" extreme selection whereby only very fit patients that could effort the logistic, economic and social difficulties related to early phase trials were referred, even in the pre-pandemic period. In this type of patient, the major exposition risk derived from a hypothetical inclusion in a phase I/II study (*i.e.*, more visits and travel necessity to other medical centres) could be overcome by the hypothetical benefit derived from these studies.

In our opinion, interesting findings of our survey are enclosed in the fourth questions' group. In this part, responders highlighted the necessity to implement the "early phase system" sharing possible solutions to make it more effective. The vast major-

ity of responders identified the necessity of an "alliance" between the leader and "satellite" centres as a strategy to improve early-phase trial followed by the necessity of a homogeneous distribution of phase I centre on national territory and the suggestion of greater use of telemedicine. These suggestions emphasize the necessity of implementing this system in our country, which can increasingly offer valid therapeutic alternatives (18), making it more efficient even in crisis situations.

Our survey has some limitations. It focused only on the aptitudes and practices of Italian oncologists toward early phase trials, which may explain the relatively low response rate. This could also explain the highest percentage of responders that work in a phase I Unit (35,7%). Moreover, the survey was compiled by oncologists working in different regions with heterogeneous hospital organizations and a different COVID-19 outbreak spread.

Nevertheless, the lack of significant differences in the aptitudes and practice between oncologists' subgroups highlights the global impact of this health care emergency irrespective of the actual burden of the COVID-19 outbreak in each respondent's hospital. Moreover, the necessity to implement early phase trials and some of the proposed solutions are also shared by other researchers (19, 20).

In conclusion, this survey provides evidence of the impact of the COVID-19 outbreak on aptitude at referral to early phase clinical studies among Italian oncologists. We highlighted the necessity to introduce many operational efficiencies in clinical trials some of which were already implemented to face the COVID pandemic. However, this is an opportunity to make permanent improvements in clinical trials, even in early phase clinical trials.

ETHICS

Fundings

There were no institutional or private fundings for this article.

Conflict of interests

AP worked at AstraZeneca Medical Affair Division from March 2015 to December 2018 and received personal fee from GSK. SD worked at AbbVie Medical Affair Di-vision from July 2017 to March 2020. GS has served on advisory boards for TESARO Bio Italy S.r.l, Johnson & Johnson, Clovis Oncology Italy S.r.l. He received support for travel or accommodation from MSD Italy S.r.l and Clovis Oncology Italy S.r.l, and institutional research funding from MSD Italy. S.r.l. GD has served on advisory board of Beigene and received sup-port for travel and accommodation from Roche. PL, RF, MF, VA, RG and FPS indicated no conflicts of interests.

Availability of data and materials

Available just before a reasonable request.

Authors' contribution

Conceptualization, PL, RF and MF; methodology, GD; formal analysis, PL; writing original draft preparation, PL, RF, MF and VA; writing review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Ethical approval

N/A.

Consent to participate

N/A.

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SUPPLEMENTARY MATERIALS

Supplementary survey. Impact of the COVID-19 pandemic on early stage clinical trials

Early phase clinical trials (phase I and II) represent one of the most critical moments in the development of new drugs, requiring intensive clinical monitoring in the face of an uncertain benefit for the patient. With this survey we would like to evaluate the impact of the COVID-19 pandemic in the propensity to refer patients to centers that conduct early phase clinical trials and how this may affect future clinical practice.

1. How old are you?
- ☐ < 30 years
- ☐ between 30 and 45 years
- ☐ > 45 years old

2. Gender
- ☐ Man
- ☐ Woman
- ☐ Not binary
- ☐ I'd rather not answer

3. In which center do you work?
- ☐ Local Health Service
- ☐ General Hospital
- ☐ Private Clinic
- ☐ Scientific Institute for Research, Hospitalization and Healthcare (IRCCS)
- ☐ University Hospital

4. In which territorial area of Italy do you work?
- ☐ North
- ☐ Center
- ☐ South

5. Specify the region:
- ☐ Valle d'Aosta
- ☐ Piedmont
- ☐ Liguria
- ☐ Lombardy
- ☐ Trentino Alto Adige
- ☐ Veneto
- ☐ Friuli Venezia Giulia
- ☐ Emilia Romagna
- ☐ Tuscany
- ☐ Umbria
- ☐ Lazio
- ☐ Marche
- ☐ Abruzzo
- ☐ Molise
- ☐ Campania
- ☐ Basilicata
- ☐ Puglia
- ☐ Calabria
- ☐ Sicily
- ☐ Sardinia

6. What is your medical specialization?
- ☐ Oncology
- ☐ Radiotherapy
- ☐ Gynecology
- ☐ Pulmonology
- ☐ Pediatrics
- ☐ Endocrinology
- ☐ Gastroenterology
- ☐ Other:

7. Do you have a focus on a particular cancer type? (multiple choice possible)
- ☐ Breast
- ☐ Lung
- ☐ Gastro-intestinal
- ☐ Genitourinary
- ☐ Melanomas and other skin cancers
- ☐ Gynecological
- ☐ Head-neck
- ☐ Endocrinological tumors
- ☐ Rare tumors
- ☐ Pediatric tumors
- ☐ I have not a specific a specific focus

8. Do you work in an early stage clinical trials unit?
- ☐ Yes
- ☐ No

9. Before the COVID-19 pandemic, did you generally refer your patients to early stage clinical trials?
- ☐ Yes
- ☐ No

10. If "YES", to what extent?
- ☐ < 1%
- ☐ between 1% and 5%

- ☐ between 5% and 10%
- ☐ more than 10%

11. If "NO", why? (multiple choice possible)
- ☐ I am not updated on the status of early phase clinical trials
- ☐ I have no reference centers nearby
- ☐ I don't have contacts with the reference center
- ☐ I think that early phase studies have not clinically significant benefit
- ☐ Other:

12. Has the pandemic changed your attitude to referring patients to early stage clinical trials?
- ☐ Yes, I refer less patients
- ☐ Yes, I refer more patients
- ☐ No

13. If "YES, I refer less patients", why? (multiple choice possible)
- ☐ I treat less cancer patients than the pre-pandemic outbreak
- ☐ I was working in departments dedicated to the COVID-19 emergency
- ☐ There is less availability of early phase clinical trials
- ☐ More logistical difficulties for patients due to measures to limit the spread of the virus
- ☐ Early phase studies require excessive medicalization in a pandemic era
- ☐ I select more cautiously patients to refer to early phase studies
- ☐ During the pandemic the increase in the care workload has reduced the possibility of collaboration with other centers

14. If "YES, I refer less patients ", What is the entity of reduction?
- ☐ Reduction < 25%
- ☐ reduction between 25% and 50%
- ☐ reduction between 50% and 75%
- ☐ reduction > 75%

15. If "YES, I refer more patients ", why? (multiple choice possible)
- ☐ Potentially superior treatment options are available compared to the standard treatment
- ☐ Centers with early phase clinical trials have "clean" pathways
- ☐ Other:

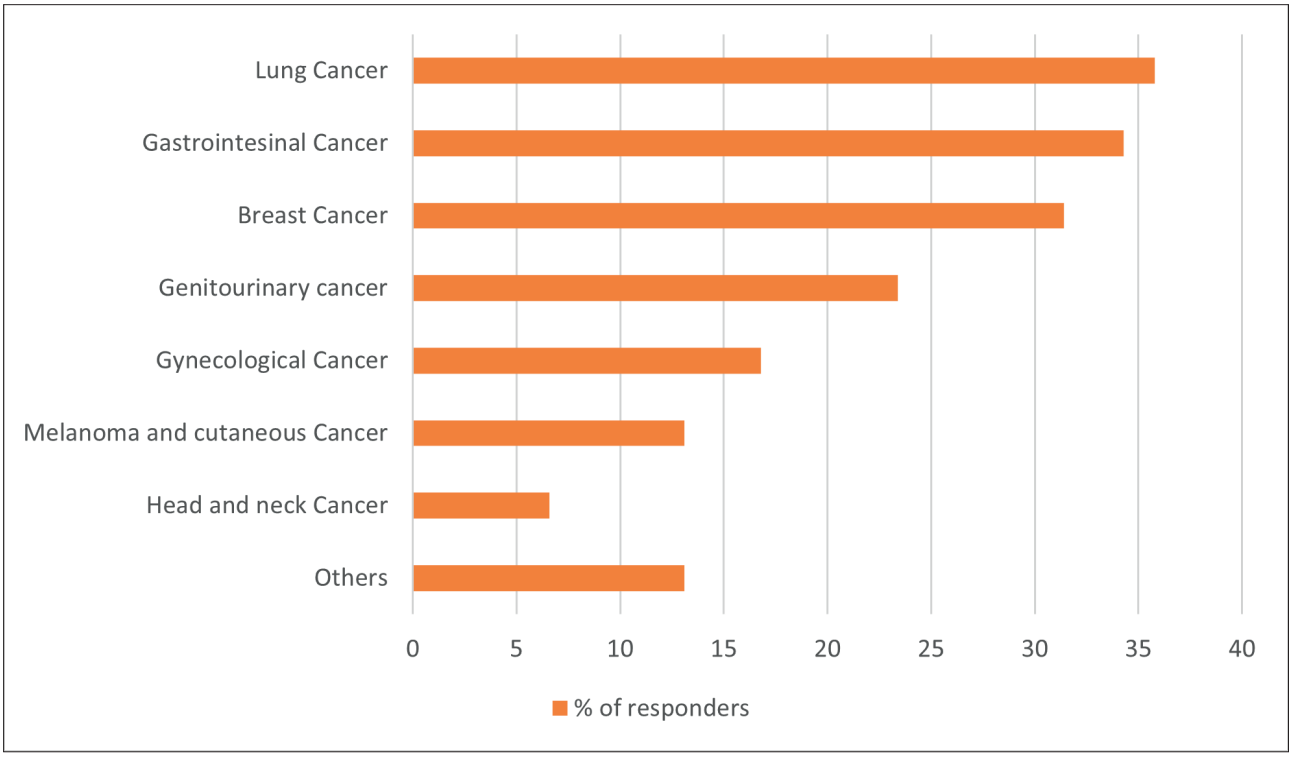
16. If "YES, I refer more patients", to what extent have you increased compared to the pre-pandemic attitude?
- ☐ increase < 25%
- ☐ increase between 25% and 50%
- ☐ increase between 50% and 75%
- ☐ increase > 75%

17. If "NO", why? (multiple choice possible)
- ☐ The pandemic has not changed my clinical practice
- ☐ The benefits of an early phase clinical trial outweigh the risks related to the pandemic
- ☐ I work in a center with an early phase Unit
- ☐ Other:

18. How long do you think it will take to return/go back to a pre-pandemic level?
- ☐ Within 6 months
- ☐ Between 6 and 12 months
- ☐ In more than 12 months
- ☐ I don't think there are any differences with the pre-pandemic period

19. How do you think we could facilitate patients' access to early phase clinical trials? (multiple choice possible)
- ☐ Thinking back to a homogeneous territorial distribution of early stage centers
- ☐ Establishing alliances between main centers and satellite centers
- ☐ By encouraging the use of telemedicine to reduce hospital visits
- ☐ Other:

20. Do you think the COVID-19 pandemic could have an impact on the development and availability of new drugs in the coming years? (multiple choice possible)
- ☐ Yes, because I believe fewer studies have been opened and we will have fewer drugs available
- ☐ Yes, because I believe that fewer patients have been enrolled and it will take longer for new drugs to become available in clinical practice
- ☐ Yes, because most of the resources have been devoted to research on COVID-19
- ☐ No, I don't think there will be an impact in the next few years



Supplementary figure 1. *Diseases of interest among responders.*

	N = 137	%
Do you think the COVID-19 pandemic could have an impact on the development and availability of new drugs in the coming years?		
No, I don't think there will be an impact in the next few years	38	27.7
Yes, because I believe fewer studies have been opened and we will have fewer drugs available	28	20.4
Yes, because I believe that fewer patients have been enrolled and it will take longer for new drugs to become available in clinical practice	44	32.1
Yes, because most of the resources have been devoted to research on COVID-19	32	23.4

Supplementary table I.

	N = 137	%
How do you think we could facilitate patients' access to early phase clinical trials?		
Thinking back to a homogeneous territorial distribution of early stage centers	51	37.2
Establishing alliances between main centers and satellite centers	82	59.8
By encouraging the use of telemedicine to reduce hospital visits	38	27.7

Supplementary table II.

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