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REVIEW

TARGETED THERAPY IN CHOLANGIOCARCINOMA: CURRENT LANDSCAPE AND FUTURE HORIZON

REVIEW

FOCUSED ULTRASOUND THERAPY IN CANCER CARE

CASE REPORT

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BRIEF REPORT

THE PROFESSIONAL USE OF SOCIAL MEDIA AMONG RESEARCH PROFESSIONALS. A NATIONAL MULTIDISCIPLINARY SURVEY

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EDRA SpA

Via G. Spadolini, 7
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Tel. 0039 (0)2-88184.1
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REVIEW

TARGETED THERAPY IN CHOLANGIOCARCINOMA: CURRENT LANDSCAPE AND FUTURE HORIZON

Chiara C. **Pircher** [1,*], Caterina **Sposetti** [1], Federico **Nichetti** [1], Francesca **Corti** [1], Alessandra **Raimondi** [1], Maria Di **Bartolomeo** [1], Edeline **Julien** [2], Monica **Niger** [1]

[1] Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy
[2] Medical Oncology Department, Centre Eugène Marquis, Rennes, France
[*] Correspondence to: chiara.pircher@istitutotumori.mi.it, https://orcid.org/0000-0001-8173-824X.

ABSTRACT: Cholangiocarcinoma (CCA) is a malignant disease of the biliary tree which accounts for 15% of primary liver cancers and 3% of gastrointestinal malignancies. For almost a decade, palliative chemotherapy remained the standard of care for advanced CCA with only modest disease control and survival rates, both in first- and second-line settings. In recent years, thanks to the advances of next-generation sequencing (NGS), an extensive range of targetable genetic alterations has been identified. In this review, we highlight different driver genomic alterations and their relative frequencies in advanced CCA, and we provide an updated overview of the current targeted treatment landscape.

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Impact statement: This review highlights different driver molecular alterations and their relative frequencies in advanced CCA and provides an updated overview of the current targeted treatment landscape.

Key words: *target therapy; advanced cancer; cholangiocarcinoma; biomarkers; resistance.*

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INTRODUCTION

Cholangiocarcinoma (CCA) accounts for <3% (1) of all gastrointestinal malignancies and includes a cluster of heterogeneous tumours arising from the intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) biliary tree. CCA is a rare cancer, but its incidence (0.3–6 per 100,000 inhabitants per year (2)) and mortality (1–6 per 100,000 inhabitants per year, globally (3)) have been increasing in the past few decades worldwide, representing a global health problem.

In early stages, CCA is generally asymptomatic or presents with nonspecific symptoms, thus most patients are diagnosed when their disease is already inoperable (4). Unfortunately, prognosis for patients diagnosed with locally advanced or metastatic CCA is dismal, with a median overall survival (mOS) of only 19 and 9 months for patients with AJCC stage III and IV CCA, respectively, compared with 69 and 33 months for patients with stage I or II CCA, respectively (5).

Until recently, treatments for patients with advanced disease have relied on the use of palliative chemotherapy with only modest disease control and survival rates, both in first- and second-line settings. In recent years, the development of next generation sequencing (NGS) techniques, which can be performed both on tumour tissue and circulating blood tumour DNA (ctDNA), have identified an extensive range of molecular alterations in CCA. Genomic data suggest that up to 40-70% (6, 7) of patients with CCA harbor potentially actionable alterations (8) (**figure 1**) with different levels of evidence for clinical actionability of molecular targets according to the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT) (9). Given the accumulating evidence that advanced CCAs could benefit from treatment personalization, the European Society of Medical Oncology (ESMO) recently recommended comprehensive NGS profiling of every patient with advanced CCA, to better define the treatment strategy (9).

In this review, we highlight different driver molecular alterations and their relative frequencies in advanced CCA, and we provide an updated overview of the current targeted treatment landscape.

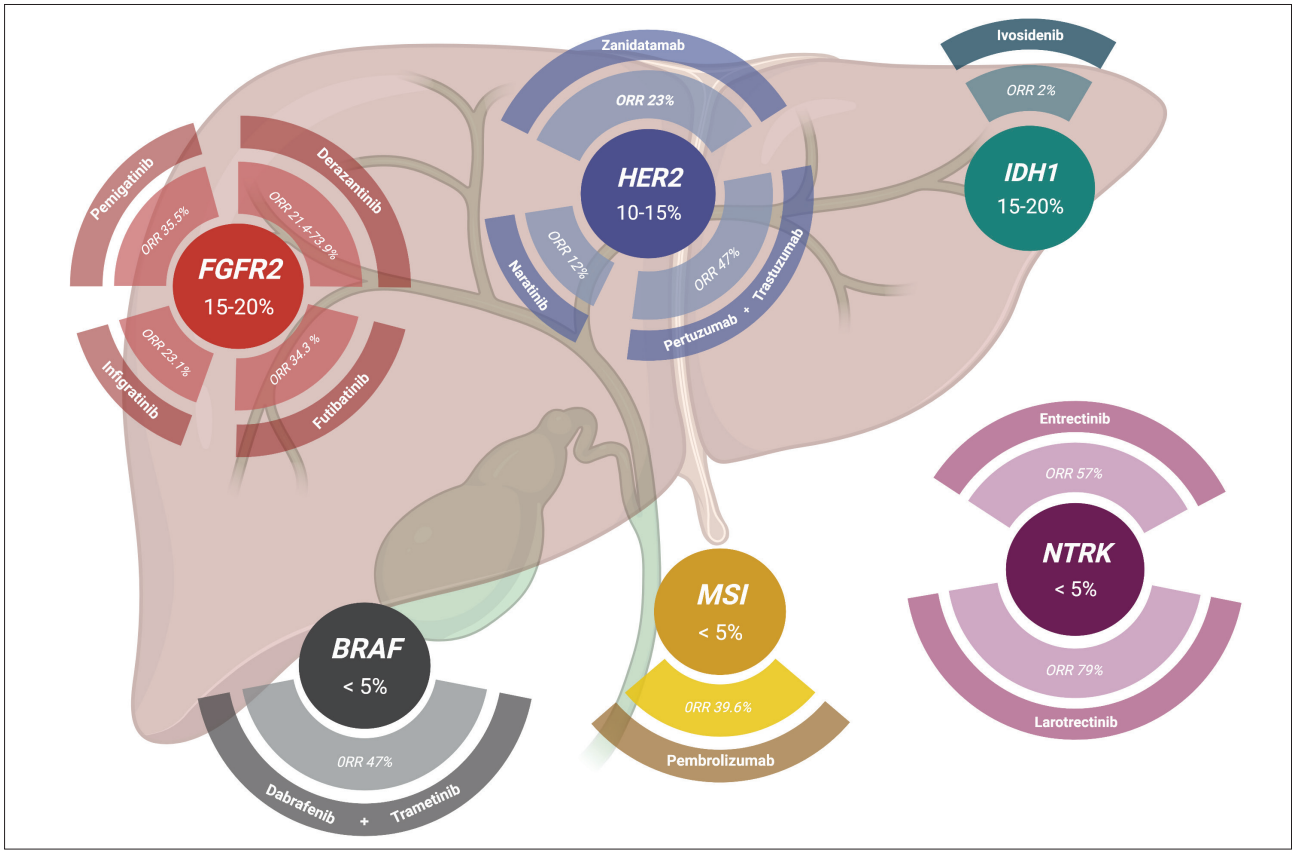

Figure 1. *Summary of relevant pathways responsible for development and progression of CCA. Red bubbles indicate molecular alterations of the gene which lead to hyperactivation or deregulation of signalling pathways, ultimately responsible for tumour growth, apoptosis evasion, proliferation, migration and invasion of cancer cells.*

GENETIC LANDSCAPE AND ACTIONABLE ABERRATIONS

Data from several studies indicate TP53, KRAS, CDKN2A/B, ERBB2, BRAF, BAP1, PI3KCA, ARID1A, IDH1/2, FGFR1–3, PBRM1, SMAD4, BRCA 1/2 and MCL1 as the most frequently altered genes in tumour tissues from patients with CCA (7, 8, 10). ARID1A, CDKN2A/B, TP53, IDH1, BAP1 and FGFR2 gene aberrations are more commonly found in iCCA compared with eCCA, while HER2 amplification and TP53 mutations are most frequent in eCCA; in detail, alterations in IDH1, BAP1 and FGFR2 are almost limited to iCCA (7, 8, 10, 11), with a trend toward mutual exclusivity between FGFR2 fusions and KRAS, IDH1 or BRAF mutations (8, 12).

Additionally, programmed Death-Ligand 1 (PD-L1) expression has also been reported to be present in 10-70% of iCCA tumor specimens (13-15) while microsatellite unstable tumors (MSI-H) are uncommon and occur only in a minority of patients (ranges from 1 to 10%) (13, 16).

Among these alterations, there are at least 4 that are considered ESCAT level I, as there is enough data to drive treatment decisions in daily practice (IDH1 mutations, FGFR2 fusions, NTRK fusions and MSI-H) (9). Furthermore, there is growing evidence for treatments targeting ESCAT level II and III alterations such as BRAF and BRCA 1/2 mutations or ERBB2 amplification. **Table I** includes targeted agents with corresponding pivotal clinical trials, efficacy, and common adverse events whereas **table II** shows promising molecularly targeted therapies currently under investigation. The most relevant pathways (**figure 2**) and drugs are discussed below.

FIBROBLAST GROWTH FACTOR RECEPTORS (FGFRS)

FGFRs consist of four transmembrane receptors (FGFR 1–4) with intracellular tyrosine kinase domains that carry out essential physiologic functions involving cell proliferation, differentiation, migration, and apoptosis via binding to their ligands (fibroblast

Table I. *Molecularly targeted therapies approved by regulatory agency or with concluded clinical trials showing relevant efficacy for CCA.*

ACTIONABLE ALTERATION	DRUG (COMPARATOR)	TRIAL (PHASE)	SETTING	PATIENT NUMBER	ORR	MPFS (MO)	MOS (MO)	MAIN AES (FREQUENCY)	APPROVAL STATUS
IDH1 mutation	Ivosidenib (placebo)	ClarIDHy (III)	≥2nd line, stage IV CCA	187	2%	2.7	10.3	nausea (41%), diarrhea (35%), fatigue (31%)	Approved by FDA (2021)
FGFR2 fusion or rearrangement	Pemigatinib (none)	FIGHT-202 (II)	≥2nd line, stage IV CCA	108	37%	7.0	17.5	hyperphosphatemia (60%), alopecia (49%), diarrhea (47%)	Approved by FDA (2020), EMA (2021)
	Infigratinib (none)	NCT0215096 (II)	≥2nd line, stage IV CCA	108	23%	7.3	12.2	hyperphosphatemia (77%), eye disorders (68%), stomatitis (55%)	Approved by FDA (2021)
	Futibatinib (none)	FOENIX-CCA2 (II)	≥2nd line, stage IV CCA	103	42%	9.0	21.7	hyperphosphatemia (85%), alopecia (33%), dry mouth (30%)	Priority review by FDA
FGFR alteration	Erdafitinib (none)	NCT02699606 (II)	≥2nd line, stage IV CCA	22	41%	5.6	40.2	dry mouth (68%), stomatitis (64%)	Not approved
NTRK fusion	Larotrectinib (none)	LOXO (I), NAVIGATE (II) [pooled analysis]	stage IV solid tumor, no standard treatment available	153 *	79% *	28.3 *	44.4 *	increased AST/ALT (45%), anemia (45%), fatigue (37%) *	FDA (2018), EMA (2019)
	Entrectinib (none)	ALKA (I), STARTRK-1 (I), STARTRK-2 (II) [pooled analysis]	stage IV solid tumor, no standard treatment available	54 *	57% *	11.0 *	21.0 *	increased creatinine (73%), anemia (67%), hyperuricemia (52%) *	FDA (2019), EMA (2020)
BRAF V600E mutation	Dabrafenib + Trametinib (none)	ROAR (II)	stage IV BTC, no standard treatment available	43	51%	9.0	14.0	pyrexia (60%), nausea (42%), fatigue (33%)	Not approved
dMMR/MSI	Pembrolizumab (none)	KEYNOTE-158 (II)	≥2nd line, stage IV noncolorectal tumor	233 *	34% *	4.1 *	23.5 *	fatigue (14%), pruritus (13%), diarrhea (12%)	FDA (2017), EMA (2022)
TMB-H (≥10 mut/Mb)	Pembrolizumab (none)	KEYNOTE-158 (II)	≥2nd line, stage IV noncolorectal tumor	102 *	29% *	2.1 *	11.7 *	fatigue (16%), hypothyroidism (12%), pruritus (10%)	FDA (2020)
ERBB2/HER2 amplification or overexpression	Trastuzumab + Pertuzumab	MyPathway (II)	≥2nd line, stage IV BTC	39	23%	4.0	10.9	diarrhea (33%), increased AST/ALT (31%), anemia (20%)	Not approved
EBB2/HER2 activating mutation	Neratinib	SUMMIT (II)	≥2nd line, stage IV BTC	25	16%	2.8	5.4	diarrhea (56%), vomiting (48%), fatigue (40%)	Not approved
ERBB2/HER2 amplification or overexpression	Zanidatamab	NCT02892123 (I)	≥2nd line, stage IV BTC	20	47%	-	-	diarrhea (43%), infusion-related reactions (33%)	Not approved
HER2-positive	Trastuzumab deruxtecan	JMA-IIA00423	≥2nd line, stage IV BTC	22	36%	5.1	7.1	anemia (53.1%), neutropenia (31.3%), and leukopenia (31.3%), interstitial lung disease (25%)	Not approved
HER2-low-expressing				8	12%	3.5	8.9		

** Data related to overall trial population of patients with an advanced solid tumor, not only BTC.*

BTC: biliary tract cancer; CCA: cholangiocarcinoma; EMA: European Medicines Agency; FDA: Food and Drug Administration; dMMR: deficient mismatch repair; mOS: median overall survival; mPFS: median progression-free survival; MSI: microsatellite instability; ORR: objective response rate; TMB-H: high tumor mutational burden.

Table II. *Main molecularly targeted therapies currently under investigation for CCA.*

ACTIONABLE ALTERATION	DRUG (COMPARATOR 1) [COMPARATOR 2]	TRIAL (PHASE)	SETTING	ORR (%) *	MPFS (MO) *	MOS (MO) *
FGFR2 fusion or rearrangement	Pemigatinib (GemCis)	FIGHT-302 (III)	1st line, stage IV CCA	-	-	-
	Infigratinib (GemCis)	PROOF-301 (III)	1st line, stage IV CCA	-	-	-
	Futibatinib (GemCis)	FOENIX-CCA3 (III)	1st line, stage IV CCA	-	-	-
	Derazantinib + Atezolizumab (none)	NCT05174650 (II)	2nd line, stage IV iCCA	-	-	-
FGFR2 fusion, mutation or amplification	Derazantinib (none)	FIDES-01 (III)	≥2nd line, stage IV iCCA	8.7%	7.3	-
IDH1 or IDH2 mutations	LY3410738 (none)	NCT04521686 (I)	1st line, stage IV CCA	-	-	-
	Vorasidenib (none)	NCT02481154 (I)	≥1st line, stage IV CCA	-	-	-
	Ceralasertib + Olaparib (none)	NCT03878095 (II)	≥2nd line, stage IV CCA	-	-	-
	Olaparib + Durvalumab (none)	NCT03991832 (II)	1st–3rd line stage IV BTC	-	-	-
KRAS G12C mutation	Adagrasib (none)	KRYSTAL-1 (I/II)	≥2nd line, stage IV solid tumor	41%	6.6	-
HRD	Olaparib	NCT04042831 (II)	≥2nd line stage IV BTC	-	-	-
[I-O]	Pembrolizumab + Lenvatinib (none)	LEAP-005 (II)	≥2nd line stage IV BTC	10%	6.1	8.6
	Pembrolizumab + GemCis (placebo + GemCis)	KEYNOTE-966 (III)	1st line stage IV BTC	-	-	-
	Durvalumab + GemCis (placebo + GemCis)	TOPAZ-1 (III)	1st line stage IV BTC	27%	7.2	12.8
	Nivolumab + Ipilimumab (none)	CA209-538 (II)	≥1st line stage IV BTC	24%	3.1	6.1
	Nivolumab (none)	NCT02829918 (II)	≥2nd line stage IV BTC	22%	3.7	14.2
	Nivolumab + Gem Cis (Nivolumab + Ipilimumab)	NCT03101566 (II)	1st line stage IV BTC	-	-	-
	Tremelimumab + GemCis (Durvalumab + GemCis) [Tremelimumab + Durvalumab + GemCis]	NCT03046862 (II)	1st line stage IV BTC	50% (73%) [73%]	13 (11) [12]	15 (18) [21]

** Ongoing studies, preliminary results reported when available.*

HRD: homologous recombination deficiency; I-O: immune-oncology; mOS: median overall survival; mPFS: median progression-free survival; ORR: objective response rate.

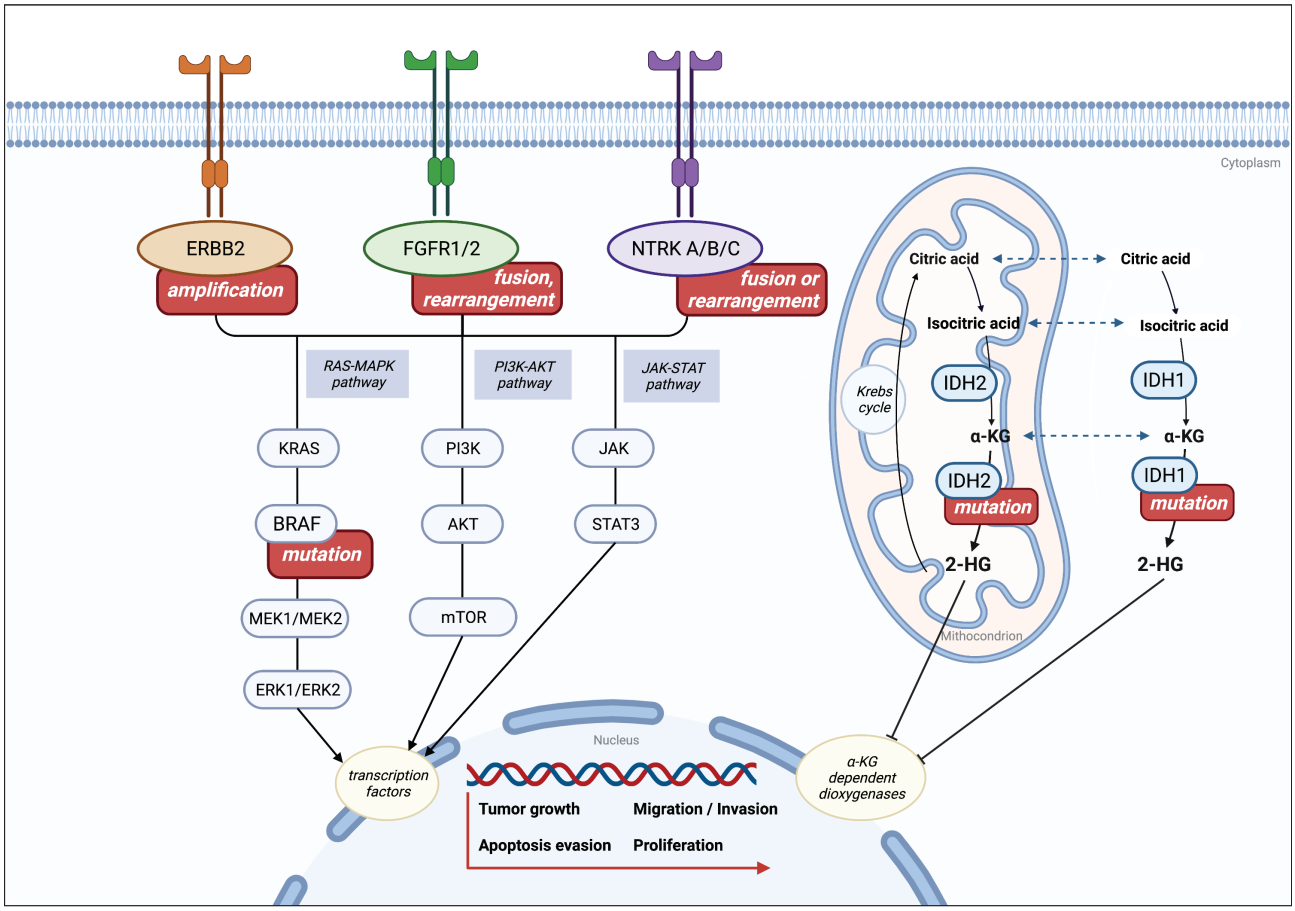

Figure 2. *Main targets of molecular therapies in CCA. Each sphere shows the relative frequency of targetable alterations. In semi-circles are shown therapies with relative objective response rate (ORR). MSI, BRAF and NTRK alterations represent agnostic target which are relatively rare in BTC, while FGFR2 fusion/rearrangements and IDH1 mutations are peculiar of iCCA and ERBB2/HER2 amplification/overexpression is frequent in every type of CCA. ORRs of Larotrectinib and Entrectinib refers to overall population of patients with advanced solid tumor harboring a NTRK fusion or rearrangement.*

growth factors, FGFs) (17). FGFRs aberrations are mostly located in the gene coding for FGFR2, with a minority of FGFR1 alterations (0.9% [FGFR1] *vs*. 6.1% [FGFR2] *vs*. 0% for the other family members), and there is a predominance of rearrangements or fusions (3.5%) over amplifications (2.6%), while mutation events are rare (0.9%) (18). FGFR2 fusion proteins are activated by the spontaneous dimerization of their respective partners, thus inducing the activation of downstream oncogenic signalling pathways including RAS-RAF-MEK-ERK/MAPK, PI3K/AKT/mTOR and JAK/STAT pathways.

Interestingly, FGFR2 fusion-positive tumours are almost exclusively found in iCCAs with an incidence of 10-20%, mainly in non Opistorchis Viverrini (11, 19) related iCCA and in females (20) and seems to be associated with indolent disease, even if the impact on prognosis is yet to be confirmed (21, 22).

Several FGFR inhibitors are currently being developed, many of which have already shown adequate safety and early efficacy in phase I-II studies for heavily pre-treated patients with iCCA harbouring an FGFR2 fusion; recent data show efficacy of FGFR inhibitors also across a broader spectrum of FGFR aberrations like FGFR2 mutations or amplification or uncharacterized FGFR1-3 aberrations (23, 24).

Currently, three of these agents are moving into phase III clinical trials, exploring their potential role in the first-line setting compared to standard Cisplatin and Gemcitabine (CisGem) chemotherapy, but the trials experience some difficulties of accrual.

Pemigatinib

Pemigatinib (INCB054828) is a selective, reversible, oral inhibitor of FGFR 1-3. Its efficacy and safety were assessed in a single-arm phase 2 trial (FIGHT- 202) of patients with previously treated locally advanced/metastatic CCA with documented FGFR status (25, 26).

One hundred and seven of the enrolled 146 patients harboured FGFR2 gene fusions or rearrange-

ments. Of these, 35.5% achieved objective disease response (3 had complete responses and 35 had partial responses), and the disease control rate (DCR) was 82%. Median Progression Free Survival (mPFS) was 6.9 months and mOS was 21.1 months. Instead, no responses occurred in the groups of patients with other FGFR aberration or without FGFR alterations (26).

Based on these positive results, pemigatinib received approval by the FDA (27) and EMA for the treatment of adults with previously treated, unresectable locally advanced or metastatic CCA with an FGFR2 fusion or other rearrangement. Moreover, the antitumor activity and manageable toxicities associated with pemigatinib prompted the ongoing phase 3 clinical trial (FIGHT-302; NCT03656536) which compares pemigatinib with CisGem chemotherapy for advanced CCA patients with FGFR2 rearrangements in the first-line setting.

Infigratinib

Infigratinib (BGJ398) is an oral bioavailable, selective, ATP-competitive pan-FGFR kinase inhibitor. Its therapeutic activity was assessed in a single-arm phase II clinical trial in patients with advanced CCA containing FGFR1-3 alterations, including FGFR fusions, mutations and amplifications who have progressed on or were intolerant to cytotoxic therapy (NCT02150967). The preliminary results of 61 patients with FGFR2 alteration reported that all responsive tumour contain FGFR2 fusions; the overall response rate (ORR) was 14.8%, the DCR was 75.4% and the mPFS was 5.8 months, which is comparable to first-line chemotherapy (20, 28). Subsequently, Javle *et al.* reported an ORR of 23.1% (with one confirmed complete response) in the 108 patients with FGFR2 fusions or other rearrangements (29). Recently, based on these encouraging data, infigratinib received approval by the FDA for the treatment of adults with previously treated, unresectable locally advanced or metastatic CCA with an FGFR2 fusion or other rearrangements. Recently, a phase III random controlled trial has started recruiting subjects with CCA harboring FGFR2 gene alterations to evaluate the efficacy and safety of infigratinib *vs.* chemotherapy (NCT03773302) in the first-line setting.

Derazantinib

Derazantinib is an oral bioavailable, selective, ATP-competitive pan-FGFR kinase inhibitor which demonstrated good tolerability and single-agent activity in heavily pre-treated patients with unselected advanced tumours in a dose-escalation phase I study (30). In a *post hoc* analysis, the anti-tumour activity of derazantinib as measured by DCR and PFS seems to be similar for patients with FGFR2 fusions and FGFR2 mutations/amplifications while patients without any detectable FGFR gene aberration appear to derive no benefit (31). These data were confirmed in a subsequent phase I/II study of derazantinib in 29 FGFR2 gene fusion-positive, unresectable iCCA patients showing a mPFS of 5.7 months, an ORR of 20.7%, and a DCR of 82.8% (32).

A larger pivotal trial of Derazantinib targeting FGFR2 gene fusion-, mutation- or amplification-positive iCCA is ongoing (NCT03230318). The interim analysis suggest that Derazantinib provided clinical benefit not only in the subgroup of 103 patients with advanced iCCA harbouring FGFR2 fusion (ORR 21.4%, including 22 partial responses, DCR 74.8%, mPFS 7.8 months and mOS 15.5 months (33)) but also in the subgroup of 23 patients harbouring FGFR2 mutations/amplifications (ORR 73.9%, including 2 partial responses, mPFS 7.3 months) (24).

Futibatinib

Futibatinib (TAS-120) is an irreversible and highly selective inhibitor which targets all four FGFR subtypes (34). It is under evaluation in a phase II study (NCT02052778) of 103 biliary tract cancer (BTC) patients with FGFR2 fusion or rearrangement, of which 67 were evaluable for efficacy in a recently presented interim analysis. In this group of patients (82% harbouring FGFR2 fusions), futibatinib gained an ORR of 34.3% and a DCR of 76.1% (35). In a preliminary phase I study, although patients with iCCA harbouring FGFR2 fusion/rearrangements experienced the greater benefit, objective responses were seen also in 2 patients with FGFR mutated CCA (23). In addition, activity of futibatinib seems significant even following progression on previous FGFR inhibitors, with a response rate of 17.9% in this setting, suggesting that it may be able to overcome mechanisms of resistance (23).

Currently, futibatinib is moving into phase III clinical trial exploring the potential role in the first-line setting compared to CisGem chemotherapy [FOENIX-CCA3; NCT04093362]) for patients with FGFR2 rearrangements.

Erdafitinib

Erdafitinib is an orally bioavailable, selective pan-FGFR kinase inhibitor (36), already approved for the treatment of patients with unresectable urothelial

carcinoma harbouring FGFR2 or 3 genomic alterations (37).

Its efficacy has been assessed in adults with advanced CCA containing FGFR alterations who had failed at least one prior systemic treatment in an open-label phase IIa study conducted in China, Korea and Taiwan (NCT02699606). At interim results from this ongoing study, 15 of the 17 treated Asian patients with advanced CCA and FGFR alterations (10 FGFR2 fusion, 4 FGFR2 mutation, 1 FGFR3 fusion, and 2 FGFR3 mutation) had an evaluable response with an objective response rate (ORR) of 7/15 (47%) and a DCR of 12/15 (80%) (38).

METABOLIC PATHWAY LINKED TO IDH1/2 MUTATIONS

Isocitrate dehydrogenase (IDH) is an essential enzyme for the citric acid cycle and converts isocitrate to α-ketoglutarate (α-KG) by oxidative decarboxylation, thus providing ATP and precursors for cellular metabolism. There are 3 human isoforms of IDH (IDH1, IDH2, and IDH3), which can be found in the cytoplasm and mitochondria (39, 40). Several studies indicate that IDH1/2 genes can be interested by gain-of-function point mutations, which means that mutant IDH1 (mIDH1) and mutant IDH2 (mIDH2) become able to catalyze the conversion of α-KG to 2-hydroxy-glutarate (2-HG), a substrate which competitively inhibits over 60 dioxygenases requiring α-KG as a cofactor, such as enzymes involved in the demethylation of DNA (41, 42). In this way, the accumulation of 2-HG, which can be detected in tumor tissue and blood (43, 44) in patients with IDH1/2 mutant tumors, impairs cellular differentiation through effects on chromatin structure and DNA methylation, finally leading to tumor initiation and progression.

Mutations in IDH genes are present in up to 10-15% (IDH1) and approximately 3% (IDH2) of CCAs; mutations are particularly frequent (up to 20%) in intrahepatic cases (7, 45, 46), especially if not related to infections (43). Prognostic implications of an IDH mutation in CCA is still under debate (47-50).

Several selective inhibitors targeting tumor harboring IDH-mutant alleles have been developed. These block the function of mutant IDH1 or IDH2 at nanomolar concentrations, leading to reduced 2-HG levels. In particular, ivosidenib has been the first mIDH1-inhibitor approved by the US Food and Drug Administration (FDA), in August 2021, for treatment of adults with previously treated, locally advanced or metastatic CCA harboring IDH1 mutation. Ivosidenib (AG-120) is a potent oral mIDH1-inhibitor and initially showed good activity in a combined phase I/II study (51), where 4 partial responses (5%), a 3.8 mPFS and 13.8 mOS were observed in 73 patients with pretreated advanced IDH1-mutated CCA. Benefit for ivosidenib in this population was further addressed in the placebo-controlled randomized phase III ClarIDHy trial (52, 53), which enrolled 187 patients with previously treated, advanced (93% metastatic) mIDH1-CCA (R132C/L/G/H/S mutation variants). Patients were randomized 2:1 to ivosidenib or placebo with cross-over permitted from placebo to ivosidenib following disease progression. The ORR with ivosidenib was 2.4%; however, DCR was 50.8% and mPFS was longer with ivosidenib (2.7 months) *vs.* placebo (1.4 months). With 70% of patients who crossed over from placebo to ivosidenib, the mOS (accounting for crossover) in the placebo group was 5.1 months *vs*. 10.3 months in the ivosidenib group. Based on these results, ClarIDHy was the first phase III trial reporting positive results with targeted therapies in CCA patients, and ivosidenib is now recommended by the NCCN guidelines for second line therapy in IDH1-mutant cholangiocarcinoma. This landmark study resulted in FDA approval of ivosidenib in this population of patients and mandates the provision of molecular profiling in CCA (53).

Several other mIDH1/2-inhibitors that might be effective in CCA are still undergoing testing in clinical trials (see **table II**).

The combination of mIDH-inhibitors with first-line chemotherapy may represent another step in improving the efficacy of this drugs if safety profile is acceptable. For instance, a phase I/II trial is studying the combination of an oral selective mIDH1-inhibitor, Olutasidenib (FT-2102), with standard first-line chemotherapy (CisGem) in patients with advanced IDH1-mutated iCCAs (NCT03684811).

Furthermore, in preclinical studies, IDH1/2-mutated tumor cells show an impaired mechanism of homologous recombination, which makes them vulnerable to poly ADP ribose polymerase (PARP) inhibitors (54). Indeed, several phase I/II trials are actually investing the efficacy of PARP-inhibitors in the treatment of advanced pretreated solid tumors with IDH1/2 mutations, including CCA, alone (NCT03212274; NCT03207347) or in combination with other agents affecting related pathways, such as ataxia telangiectasia and rad3 related (ATR) inhibitors (NCT02576444;

NCT03878095) or immune checkpoint inhibitors, such as durvalumab (NCT03991832).

Finally, there is currently focus on the biological rational of combining IDH inhibition with immunotherapy, in particular with anti-CTLA4 antibodies, in relation to immunosuppressive effects mediated by 2-HG and to the preclinical evidence that treatment with mIDH inhibitors results in the simultaneous recruitment of effector and immunoregulatory cells: thus, additional CTLA4 blockade may favor the immune effector response, resulting in synergistic antitumor effect (55, 56).

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)/HER2

The HER family receptors consist of four distinct receptors: epidermal growth factor receptor (EGFR) or HER1, HER-2, HER-3, and HER-4, corresponding to the EGFR, ERBB1/2/3/4 genes. In normal cells, binding of the ligands to the extracellular domain of these receptors leads to the dimerization with eventual phosphorylation of the intracellular tyrosine kinase domain and activation of the downstream pathways, which include MAPK, PI3K/AKT/mTOR, and STAT pathways that control and regulate cell proliferation, differentiation, and metabolism (57). In CCA as well as in various human cancers, EGFR/ERBB2 alterations commonly lead to EGFR and HER2 overexpression which is highly related to tumorigenesis, tumour cell invasion and metastasis. Notably, 5-15% of CCA patients could harbour EGFR mutations (58), whereas ERBB2 amplification, overexpression or both are observed in approximately 17% of extrahepatic and 5% of intrahepatic CCA (45).

EGFR Inhibitors

Several clinical trials have recently evaluated the role of EGFR-targeted drugs, usually divided into EGFR tyrosine kinase inhibitors (EGFR-TKIs) and monoclonal antibodies targeting EGFR (EGFR-mAbs), but in mostly in unselected populations. After the modest benefit showed in phase II studies, a phase III study compared the efficacy and safety of erlotinib plus gemcitabine and oxaliplatin (GEMOX) regimen with GEMOX regimen alone in therapy-naive patients with advanced BTC. Patients treated with chemotherapy plus erlotinib achieved a higher ORR (29.6% *vs*. 16.5%) but mOS (9.5 months in both groups) and mPFS (5.8 *vs*. 4.2 months) did not improve significantly; however, in a subgroup analysis, patients with CCA had an improved mPFS with the combination therapy (5.9 months *vs*. 3.0 months; p = 0.049) (59). In addition, two trials evaluated the efficacy of anti-EGFR antibodies cetuximab (in patients not selected for molecular alterations with sensitivity) and panitumumab (in patients with KRAS wild type tumours) plus gemcitabine-platinum doublets with disappointing results (60, 61).

Thus, based on this previous evidence, anti EGFR inhibitors do not seem to be a promising treatment option for patients with BTC.

HER2 Inhibitors

MyPathway, a phase IIa multiple basket study, evaluated the combination of HER2-directed monoclonal antibodies pertuzumab and trastuzumab in 39 patients with previously treated BTC harbouring ERBB2 gene amplification or protein overexpression. After a median follow-up of 8.2 months, 9 patients had an objective response (all partial response), yielding an ORR of 23% (62).

Studies with other dual EFGR and HER2 tyrosine kinase inhibitors, including Afatinib and Lapatinib, failed to demonstrate the therapeutic effects in CCA patients (63-65). Preliminary results of the phase II SUMMIT basket trial testing Neratinib, an oral tyrosine kinase inhibitor of EGFR, HER2, and HER4, in pre-treated patients affected by solid tumours with activating somatic ERBB2 mutations showed, among the 25 evaluable patients, an ORR of 12%, a clinical benefit rate of 20%, a mPFS of 2.8 months and an OS of 5.4 months (66).

Furthermore, a phase I study of Zanidatamab, a bispecific HER2-targeted antibody, demonstrated among 17 evaluable patients an ORR of 47% and a DCR of 65% (67). Zanidatamab is now under further investigation as treatment option following standard-of-care for patients affected by ERBB2 amplified BTC in a phase II multicentric trial (NCT04466891).

Finally, the HERB trial evaluated the safety and efficacy of Trastuzumab deruxtecan, an antibody-drug conjugate composed of a humanized monoclonal anti-HER2 antibody and a topoisomerase I inhibitor, in patients with HER2-expressing (HER2-positive: IHC3+ or IHC2+/ISH+, and HER2-low-expressing: IHC/ISH status of 0/+, 1+/-, 1+/+, or 2+/-) BTC. Among 22 treated cases, ORR was 36% and DCR 82%, with mPFS and OS of 5.1 months and 7.1 months, respectively. In addition, encouraging effi-

cacy data were seen even in the 8 patients with low HER2 expression. Concerning safety, 8 patients (25%) had interstitial pulmonary disease, two of which were life-threatening events (68).

RAS/RAF/MEK/ERK SIGNALING PATHWAY INHIBITORS

The RAS/RAF/MEK/ERK signaling cascade is an oncogenic pathway that is activated in several cancer types, including CCA, and promotes proliferation of cancer cells, migration, and metastasis. The frequency of detected KRAS mutations for intrahepatic and extrahepatic CCA ranges from 9% to 45% and from 15% to 67%, respectively, and 4% and 3% for NRAS (69-71). Although RAS mutations serve as oncogenic drivers in many different types of malignancies, targeting RAS has mostly been unsuccessful because of its intricate interactions with other signalling pathways. Consequently, inhibiting targets downstream of RAS, such as BRAF and MEK, has been attempted in various diseases.

BRAF mutations have been described in less than 5% of patients with CCA, primarily in the cohort with intrahepatic disease (7, 72). Dual targeting with dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) within the ROAR trial, a phase II basket trial of 178 patients harbouring the BRAF V600E mutations, provided promising result. Among 43 patients with BRAF V600E-mutated BTCs (of which 91% were cholangiocarcinoma), this regimen achieved an ORR of 51%, a mPFS of 7.2 months and a mOS of 11.3 months (73).

Selumetinib, a MEK inhibitor, has been studied in combination with CisGem in a phase Ib study (ABC-04) in therapy-naive patients with advanced BTC, unselected for molecular alterations (74). Among eight evaluable patients, three had a partial response, and five had stable disease with a median PFS of 6.4 months; the most frequent adverse event were G1-2 oedema and rash. A phase II study with selumetinib, which did not select patients for molecular alterations, included 39% of chemotherapy-refractory patients, showing an ORR of 12%, a median PFS of 3.7 months, and a median OS of 9.8 months (75).

Recently, Adagrasib, a KRAS G12C-selective covalent inhibitor, demonstrated clinical activity and a manageable safety profile in pancreatic ductal adenocarcinoma and other GI cancer in a phase II study; in particular, it showed an ORR of 50% (4/8) in patients with BTC (76).

Based on these promising results, further studies are ongoing to assess the efficacy of drugs inhibiting this pathway.

NEUROTROPHIC TROPOMYOSIN RECEPTOR KINASE (NTRK)

The neurotrophic receptor tyrosine kinase (NTRK) genes, NTRK1, NTRK2, and NTRK3, which are reported as altered in less than 5% of patients with iCCA (6, 77), encode the tropomyosin receptor kinases (TRK), a receptor family composed of three transmembrane proteins: TRKA, TRKB and TRKC (78). In a normal state, TRK receptor signalling has a role in neuronal development, function, survival, and proliferation whereas intrachromosomal rearrangements of NTRK1–3 resulting in gene fusions may activate signal transduction leading to oncogenesis (79). Point mutations and amplifications of NTRK genes have also been reported in human tumours (80).

Several small-molecule TRK inhibitors are currently in clinical development. In particular, two agents have recently received FDA and EMA approval for the tumour agnostic treatment of patients with solid tumours harbouring an NTRK gene fusion; these are the selective TRK inhibitor Larotrectinib and the TRK/ROS1/ALK multikinase inhibitor entrectinib, which have yielded an impressive ORR of 57% to 75% in advanced solid tumours harbouring NTRK fusions (the percentage of patients with cholangiocarcinoma enrolled in the two pivotal trials were 4% and 2%, respectively) (81, 82). Of note, TRK inhibitors have activity also against ROS1 and ALK fusions, which are reported in 0-8.7% and 2.7% of patients with CCA, respectively (6, 83).

PI3K/AKT/mTOR PATHWAY

PI3K/AKT/mTOR is an intracellular signalling pathway in which the tumour suppressor gene PTEN plays an essential regulatory role (84). It encodes the PTEN protein, a phosphatase which reverses the actions of PI3K to control the level of PIP3, thus modulating the activation of the PI3K/Akt/mTOR pathway (85). Various types of tumours are interested by hyperactivation of PI3K or inactivation of PTEN, which results in deregulation of this signalling pathway, conferring cells oncogenic potential (86).

According to several studies, PI3K mutations were detected in 4.4% of iCCA patients and 6.5% of eCCA patients. PTEN mutations were observed in 4.4% of iCCAs and 3.9% of eCCAs (87). Blocking this sig-

nalling pathway might inhibit tumour growth. As a result, many inhibitors targeting the effector proteins in the the PI3K/Akt/mTOR pathway are under development and investigation.

Copanlisib (BAY 80–6946) is a selective pan-class I PI3K inhibitor which has been evaluated in a phase I study with 50 patients with advanced solid tumours not selected for molecular alterations, including 23 BTC patients (NCT01460537). Response rate was 6.3% in the copanlisib with gemcitabine and 12% in the copanlisib with CisGem arm. Among BTC patients, response rate was 17% (88). Currently, the therapeutic effects of the copanlisib plus CisGem regimen is being assessed in a phase II trial with CCA patients (NCT02631590).

The mTOR inhibitor everolimus has been evaluated in several clinical studies. A phase I study assessing the safety and antitumor activity of everolimus in combination with gemcitabine enrolled in 10 BTC patients, with 6 patients achieving stable disease (60%) (89). Subsequently, a phase II clinical trial was conducted to evaluate the therapeutic efficacy and safety of everolimus in 39 advanced BTC patients who were previously treated with chemotherapy. It reported a DCR of 44.7%, ORR of 5.1%, mPFS of 3.2 months and a mOS of 7.7 months (90). Another relevant phase II clinical trial of everolimus in cancer patients with PI3K mutation or PTEN loss did not show any general clinical benefit, though the only CCA patient achieved stable disease (91). In recent years, the data of the RADiChol study, a phase II clinical trial, was published (NCT00973713). Twenty-seven patients with advanced BTC were enrolled in this study. The primary endpoint DCR at 12 weeks was 48%, with a mPFS of 5.5 months and mOS of 9.5 months (92). Currently, the therapeutic efficacy of everolimus in BTC treatment has not been confirmed, so more clinical data are expected to be released.

DDR AND BRCA1/2 MUTATIONS

Poly adenosine diphosphate-ribose polymerase inhibitors (PARPi) represent a novel therapeutic option for cancer patients harbouring germline and somatic aberrations in DNA damage repair (DDR) genes. Across BTC patients, BRCA1/2 mutations are rare (1-7%) but a broader spectrum of DDR gene alterations is reported in 28.9-63.5% (93, 94). A retrospective analysis by Golan *et al.* included 18 patients, 5 with germline BRCA1/2 mutations and 13 with somatic mutations; interestingly four stage III-IV patients were treated with PARP inhibitors with an OS from 11 to 65 months (95). To date there is no evidence regarding the efficacy of PARP inhibitor in BTC patients harboring DDR gene alteration, but phase II clinical trials evaluating Olaparib and Niraparib in this setting are now ongoing (96) (NCT04042831, NCT03207347).

Furthermore, there is preclinical evidence that IDH1 and IDH2 mutations could induce a homologous recombination defect similar to a "BRCAness" phenotype (97), due to the capacity of 2HG to inhibit DNA and protein demethylation leading to a hypermethylation profile (98). This hypothesis is currently being tested in several clinical trials (NCT03212274, NCT03991832, NCT03878095). Similarly, Niger *et al.* recently suggested that even MGMT may be involved in platinum-induced DNA damage response (DDR) by playing a role in the homologous recombination signaling in cancer cells, providing the rational to explore the combinations of TMZ with DDR inhibitors such as PARP and ATR inhibitors (100).

BIOMARKERS FOR CHECKPOINT INHIBITORS SENSITIVITY

The only FDA-approved immunotherapy in BTCs is pembrolizumab, an anti-PD-1 antibody, which received tissue-agnostic approval for the treatment of advanced solid tumours with DNA mismatch repair deficiency (dMMR), microsatellite instability (MSI) and high tumours mutational burden, including those of the biliary tract (<3% cases) (100-102). However, MSI-H BTC are uncommon (ranges from 1 to 10%) and other clinical trials evaluating checkpoint inhibitors as monotherapy for advanced BTC have shown controversial and overall disappointing results. Clinical benefit seems limited only to a small percentage of BTC and, with the exception of MSI, no predictive biomarker has been identified yet (13, 103). However, various trials focused on evaluating the impact of programmed Death-Ligand 1 (PD-L1) expression, which is reported to be present in 10-70% of iCCA (13-15), on responses to checkpoint inhibitors.

In the phase Ib KEYNOTE 028, which enrolled exclusively patients with positive PD-L1 tumor expression (PD-L1 ≥1%), the ORR, PFS and OS of 23 patients with previously treated BTC were 13%, 1.8 and 5.7 months respectively (104, 105). Similarly in the KEYNOTE-158, assessing the efficacy of pembrolizumab in an unselected cancer patient population, the

ORR was 5.8% (6/104), mOS 7.4 months and mPFS 2 months. In a subgroup analysis, ORR was 6.6% (4/61) and 2.9% (1/34) among PD-L1-expressers and PD-L1-non-expressers, respectively (102). Furthermore, regarding 22 dMMR/MSI patients, response rate was 40.9% (including complete response in 2 patients), mPFS 4.2 months, and mOS 24.3 months (106).

Nivolumab has also been evaluated as second-line therapy for unselected refractory BTC patients in a multicenter phase II study which demonstrated an ORR of 22% (10 of 46) with a disease control rate of 59% (27 of 46) (107); PDL-1 expression in tumors was associated with prolonged progression-free survival (HR 0.23, P < .001 with PD-L1 cutoff of 1%; HR 0.37, P = .02 with cutoff of 10%).

Of note the recent phase III TOPAZ-1 trial (108) reported, for the first time, positive results of immunotherapy plus chemotherapy as first-line treatment for advanced BTC; durvalumab plus CisGem demonstrated statistically significant prolonged OS (12.8 mo *vs*. 11.5 mo) and PFS (7.2 mo *vs*. 5.7 mo) compared with placebo plus CisGem, regardless of the level of PD-L1 expression; in detail, the HR for OS with durvalumab *vs* placebo was 0.79 and 0.86 in patients with a PD-L1 tumour positivity area (TAP) ≥1% (>1% of tumour area occupied by tumour cells with PD-L1 staining) and <1%, respectively, with only a trend towards major efficacy for PD-L1 positive cases. Moreover, overall response rate was 26.7% with chemo-immunotherapy compared with 18.7% with chemotherapy alone (P = .011). Durvalumab did not add additional toxicity to that observed with chemotherapy alone, with similar grade 3 and 4 adverse events rates (109).

Based on these data, FDA has recently approved Durvalumab plus chemotherapy for the treatment of locally advanced or metastatic bile tract cancer (110).

RESISTANCE TO TARGET THERAPY

As previously discussed, several target therapies and immunotherapies are being assessed in clinical trials for the treatment of advanced or metastatic CCA patient; however, despite evidence of initial responses and disease control, disease progression inevitably occurs in most patients and PFS were frequently dismal up to date little is known about mechanisms of resistance to these compounds and potential ways to overcome them.

Regarding primary FGFR inhibitor resistance, Silverman and colleagues demonstrated that patients with co-occurring tumor suppressor gene alterations including BAP1, CDKN2A/B, PBRM1 and TP53 had shorter median PFS on pemigatinib than those without alterations in these genes, although the numbers of patients do not allow any significant conclusions (111).

As far as secondary resistance to FGFR2 inhibition is concerned, Goyal *et al.* first reported a small case series of three patients with FGFR2 fusion-positive iCCA developing FGFR2 V565F gate-keeper mutation (and in two cases also polyclonal secondary mutations in the FGFR2 kinase domain) as a mechanism of acquired resistance to infigratinib (112). Later, Goyal *et al.* showed that sequential treatment with futibatinib after progression on infigratinib or Debio 1347 (an ATP-competitive FGFR1–3 inhibitor) led to prolonged clinical benefit from FGFR inhibition in four patients with acquired FGFR2 kinase domain mutations (113).

In addition, Wu *et al.* recently demonstrate that EGFR-dependent signaling limits the effectiveness of FGFR inhibitor therapy determining adaptive resistance in patient-derived models with FGFR2 positive cholangiocarcinoma. This supports the potential of combination treatment with FGFR and EGFR inhibitors which would suppress MEK/ERK and mTOR signaling, increasing apoptosis, and causing marked tumor regressions (114).

Primary resistance mechanisms to IDH1 inhibitors are not yet fully elucidated. The quantity of 2-HG accumulated, however, seems to play a role in the response to mIDH-inhibitors: in the ClarIDHy trial, in fact, treatment duration appeared to be longer for patients with plasma 2-HG levels below 100 ng/mL after 1 cycle of ivosidenib (53).

With respect to acquired resistance, new mutations in other genes like TP53, ARID1A, POLE, PIK3R and TXB3 or a second site mutation in IDH1 or isoform switching from mutant IDH1 to mutant IDH2 or vice versa (115) avoid the binding of ivosidenib and increase 2-HG production (116-118).

As far as immunotherapy is concerned, even if there are no definitive data, there are some reports of acquired resistance to checkpoint inhibitors due to a deregulated Wnt/β-catenin pathway, also in MSI-H cholangiocarcinoma (119).

PITFALLS OF TISSUE NGS AND POTENTIAL OF LIQUID BIOPSY

Until recently, tissue biopsies have been represented the standard not only for pathological diagnosis and molecular characterization but also, at progression, to identify mechanism of resistance. However,

the complexity of tumor heterogeneity, which may be underrepresented in a single site biopsy, the ethical considerations, and the procedural difficulties in obtaining longitudinal tumor samples, which is often inadequate for molecular profiling, represent important issues. In a recent report by Lamarca *et al.* of real-life profiling of BTC, the rate of tissue sample failure was ~25%, mainly due to insufficient tumor representation or low DNA extracted for analysis (120). Limited access to tissue can result in a restraint of personalized treatment approaches and option for patients. Developing new strategies to identify BTC and to obtain an adequate molecular profiling is still an unmet need. In this scenario, there is mounting evidence on the use of liquid biopsy as a non-invasive and repeatable method that allows for tumor molecular profiling and treatment response (121-127).

In a recently published large cohort of 1671 patients with advanced BTC profiled with Guardant360, genetic alterations were detected in cell free DNA (cfDNA) in 84% of patients. Targetable alterations detected in 44% of patients and high pre-treatment cfDNA variant allele fraction (VAF) were associated with poor prognosis and shorter response to chemotherapy and targeted therapy. Of note, cfDNA analysis was quite reliable in detecting IDH1 mutations and BRAF V600E at similar rates to tissue profiling, but it was able to identify only 18% of FGFR2 fusions (128).

This study the utility of cfDNA analysis in current management of BTC and adds up to already mentioned literature showing the role of liquid biopsy in uncovering putative mechanisms of resistance to targeted therapies (112, 119).

CONCLUSIONS

Advanced cholangiocarcinoma is a malignancy associated with poor prognosis and rising incidence worldwide. Treatment options have been historically based on platinum combination chemotherapy however, during the past decade, immuno-oncology and mutation-targeting drugs are revolutionizing the treatment of advanced CCA.

Consequently, current clinical guidelines, including ESMO, advocate for molecular characterization, which can be performed on the tumor tissue and/or on circulating tumor DNA (ctDNA), to define the best therapeutic strategy for patients affected by CCA.

However, despite these drugs are revolutionizing our clinical practice, all patients on target therapies eventually develop treatment resistance. Understanding mechanisms involved in targeted treatment resistance and how they evolve after exposure to each drug represents a compelling and urgent need to develop new rational combination strategies. In addition, there is a lack of reliable biomarkers to identify the BTC patients most likely to benefit from immunotherapy.

In conclusion, despite major improvements in CCA characterization and treatment, further research is needed to elucidate resistance mechanisms to immune checkpoint inhibitors and targeted therapies in these patients. Moreover, the widespread of novel treatment options is still limited, and new accessible strategies are still to be defined in this difficult to treat tumor entity.

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Availability of data and materials

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

Authors' contributions

CP: conceptualization; CP, CS: methodology; MN, EJ: validation; CP, CS, FN: writing - original draft preparation; CP, CS, FN, FC, AR: writing, review and editing; FN, MdB, MN, EJ: supervision. All Authors have read and agreed to the published version of the manuscript.

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REVIEW

FOCUSED ULTRASOUND THERAPY IN CANCER CARE

Gaetano **Romano** [1, *], Elisa **Ventura** [1, 2], Kailey Shanti **Abraham** [1], Francesca **Cersosimo** [1, 2], Antonio **Giordano** [1, 2]

[1] College of Science and Technology, Temple University, Philadelphia, PA, USA
[2] Sbarro Health Research Organization, Philadelphia, PA, USA
[*] Correspondence to: gromano@temple.edu, https://orcid.org/0000-0002-6340-5879.

ABSTRACT: Focused ultrasound (FUS) holds great therapeutic potential in cancer care. Both clinical and preclinical studies indicate that FUS may eventually become a safe and efficient approach for the treatment of oncological patients. FUS-induced anticancer responses comprise tumor growth inhibition, enhanced T lymphocytes infiltration within the tumor mass and increased permeability of the vascular system, including the temporary opening of the blood-brain barrier. On these grounds, FUS can be utilized to target the tumor microenvironment, increase the efficiency of therapeutic agents and, possibly, elicit local and/or systemic host immune responses against malignant tissues and/or cells.
Clinical and preclinical studies are currently in progress to optimize anticancer FUS applications in establishing long-term systemic host immune responses, which may ultimately lead to abscopal effects in patients.

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Impact statement: This article discusses the latest developments in the field of FUS in cancer care, in terms of technology and therapeutic effects that were reported among various oncological patients. In addition, the article focuses on clinical and preclinical studies that aim at characterizing the effects of FUS on the tumor microenvironment, the impact on the vascular system and modulation of host immune responses against malignant cells.

Key words: *Focused Ultrasound Therapy; abscopal effects; radiation therapy; cancer immunotherapy; CTLA4; PD1; PDL1; PDL2; immunomodulators; immunogenic cell death.*

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INTRODUCTION

Ultrasound techniques for therapeutic applications must utilize higher energy than ultrasound for diagnosis and/or imaging, otherwise the biological effects will be obviously negligible (1). Focused ultrasound (FUS) has been designed to propagate mechanical waves within a restricted volume to produce high energy density, which is then absorbed by the tissues of the site of the treatment (1, 2). The mechanical waves are generated by a focused transducer (**figure 1**). The biological effects induced by FUS depend on the type and location of the tissue or organ that is irradiated and a variety of ultrasound parameters (3-8). In addition, gas-filled therapeutic microbubbles or nanodroplets may be added to increase the efficiency of the ultrasound-based intervention (9-11).
The main FUS parameters that can be varied to optimize the biological effects in the treated tissue are frequency, treatment time, pressure, and duty cycle (12, 13): frequency is directly proportional to the energy that is imparted into the irradiated tissue and represents the number of sound wave cycles transmitted over time. Frequency is measured in hertz (cycles per second).
Treatment time constitutes the total period that is required to scan the entire area for the intervention.
Ultrasound pressure is measured in Pascals and depends on the perpendicular force that is exerted on a determined surface area. The ultrasound pressure equals the sound wave amplitude. High pressure is utilized for ablative treatments, whereas non-ablative treatments necessitate low ultrasound pressure (7).
Duty cycle is defined as the time percentage of the on-phase ultrasound application, during the total on-and-off pattern. Thermal-based treatments require long pulses because ultrasound waves are

quickly absorbed by the irradiated tissues in the form of heat. Conversely, non-thermal treatments are conducted with short and rapid pulses to prevent the release of heat (8).

The four ultrasound parameters may be utilized in several combinations to induce different types of thermal and/or mechanical stress in treated tissues or organs.

There are four modalities for FUS-based treatments, which can be classified as follows: I) thermal ablation for the induction of coagulative tissue necrosis; II) hyperthermia and thermal stress to generate a mild cell heating without inducing coagulation; III) mechanical stimulation without the production of thermal effects; IV) histotripsy for the mechanical destruction of the irradiated tissue. Interestingly, FUS allows for the immunomodulation of the tumor microenvironment (2-5) and enhances the vascular system permeability (14, 15). These two effects may result in local and/or systemic host immune responses against the tumor and enhance the efficiency of the administration of various therapeutic agents (16, 17). On these grounds, FUS is emerging as a promising technology for the treatment of patient with cancer.

FUS-based cancer therapy is a rather recent area of investigation, which is still addressing several challenges to define the conditions for an optimal antitumor response in patients (1-11) (**table I**). FUS has a wide variety of parameters that must be adapted for each tumor type and treatment site (3-8). Furthermore, each of the previously mentioned FUS modalities for cancer therapy are associated with a particular combination of immunological and vascular effects in tumors (2-5, 14, 15). In addition to an increase in the vascular permeability, FUS-induced responses against a malignancy include enhanced T cell infiltration in malignant tissues and inhibition of tumor growth (18-23).

Studies are currently underway for the characterization of FUS-induced effects on the tumor microenvironment, which are the surroundings that support the malignant mass (18-23). The tumor microenvironment is constituted by the stroma, extracellular matrix, signaling molecules, blood and lymphatic vessels, cells of the immune system and fibroblasts (24, 25). Immunologically speaking, the tumor microenvironment can be classified as either permissive or not permissive to the infiltration of cytotoxic T lymphocytes (19, 20). In a tumor microenvironment that is permissive to the infiltration of cytotoxic T lymphocytes, the tumor-associated antigens are easily available to the host immune system. Therefore, the exposure to the host immune system confers a good degree of immunogenicity to the tumor microenvironment. Conversely, in the absence of cytotoxic T lymphocytes, the tumor microenvironment is scantily immunogenic, despite the presence

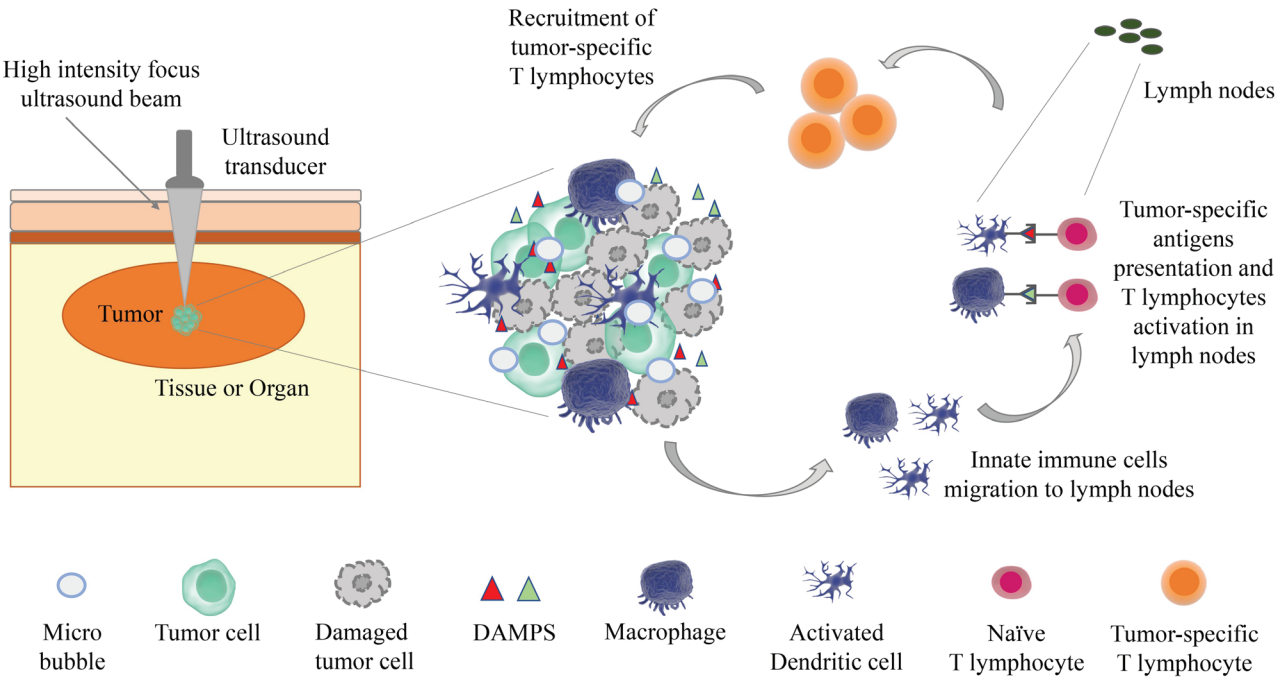

Figure 1. *Effects of an ultrasound transducer on the tumor mass. The transducer conveys the ultrasonic waves to a restricted volume of the tumoral tissue. The ultrasound acoustic waves, possibly combined with microbubbles, induce the death of malignant cells, which may elicit host immune responses against tumor associated antigens. These immunological effects are explained in greater detail in figure 3.*

Table I. *Summary of FUS modalities for the treatment of cancer.*

FUS MODALITY	BIOLOGICAL EFFECT(S)	COMBINATION OF FUS PARAMETERS TO OPTIMIZE THE FUS MODALITY
Thermal ablation. High Intensity Focused Ultrasound (HIFU).	Induction of coagulative tissue necrosis; removal of malignant tissues, without affecting healthy tissues and organs.	High pressure of sound waves transmission combined with intense duty cycle. The HIFU biological effects can be enhanced with microbubbles, or nanodroplets.
Hyperthermia and thermal stress.	Mild cell heating without inducing coagulation.	Hyperthermia and thermal stress temperature vary from 40 °C to 45 °C inside the treated area. Hyperthermia requires a treatment period of 30 to 90 minutes. Thermal stress is conducted in a shorter timeframe (from seconds to a few minutes).
Mechanical stimulation without thermal effects. (Mechanical perturbation). (LOFU).	Lack of thermal effects	Low to moderate pressure combined with high-duty cycle. Microbubbles, or cavitation may enhance the performance of mechanical stimulation.
Histotripsy for the mechanical destruction of the irradiated tissue. (M-HIFU, or cavitation cloud histotripsy). Boiling histotripsy.	Mechanical destruction and liquefaction of the irradiated tissue.	Very high pressure in combination with short-pulse ultrasound waves, which are in the range of microseconds. Boiling histotripsy requires longer pulses, which are still in the range of microseconds and produces boiling bubbles, and lower peak pressures than cavitation cloud histotripsy.

of other types of cells of the immune system. The absence of cytotoxic T lymphocytes in the tumor microenvironment may be related to a combination of factors, such as the constitution of the vasculature of the tumor and/or deficiency of priming and/or recruitment of CD8+ T cells (16, 17, 19-25). Recruiting CD8+ T cells may result from the activation of immune checkpoints pathways based on CTLA4, PD-1, PD-L1 and PD-L2 (16, 17), along with the release of other soluble factors like adenosine, which has immunosuppressive properties and is overexpressed in the tumor microenvironment (26-29).

FUS-BASED THERAPEUTIC APPROACHES IN CANCER CARE

FUS holds great therapeutic potential for the treatment of cancer because it is based on non-invasive practices that only require outpatient interventions, unlike most surgical interventions (30). Furthermore, the FUS treatments are very precise and do not involve the use of ionizing radiations (31, 32). Thus, FUS avoids the risk of infections, which are usually associated with surgical procedures, and it does not cause the adverse effects of ionizing radiations (31, 32).

FUS-based treatments are currently subdivided into four branches: thermal ablation, hyperthermia and thermal stress, mechanical stimulation without thermal effects and histotripsy. Each type of treatment has specific biological effects in malignant cells (1-11) and they can be produced by varying the four main parameters of FUS: frequency, treatment time, pressure and duty cycle (**table I**).

In addition, the use of gas-filled microbubbles, or nanodroplets can enhance the FUS-derived mechanical and/or thermal effects in targeted tumoral tissue (33-35). The increased efficiency of the treatment may allow for the reduction of acoustic waves intensity, along with a more precise intervention in the affected area (33-35). Gas-filles microbubbles or nanodroplets are administered via intravenous injection. The protocol for the production and purification of microbubbles and/or nanodroplets can be found at https://www.jove.com/it/t/62203/production-membrane-filtered-phase-shift-decafluorobutane. These gas-filled particles respond to the stimulation of acoustic waves with non-linear and fast oscillations, during which the acoustic radiation force (ARF) moves the oscillating microbubbles along the vascular walls. This phenomenon is termed cavitation (36, 37) and consists of an intricate series of events that derive from a variety of physical effects (38-40). Nevertheless, cavitation is simply summarized either as stable, or inertial phase (38-40). The FUS-induced gradual and constant oscillations give rise to a stable cavitation (38), whereas inertial cavitation consists of a fast expansion and violent collapse of the microbubbles in response to

FUS. Inertial cavitation produces various effects, such as: intense heat and pressure in the volume of the bubble core, presence of reactive oxygen molecules and local mini-streams that harm tissues and cells in the area of the intervention (39, 41, 42).

THERMAL ABLATION

The most common and best characterized ultrasound-based therapeutic approach is thermal ablation, which is also termed High Intensity Focused Ultrasound (HIFU) (43, 44). Thermal ablation is a noninvasive modality for the extirpation of malignant tissues that does not damage nearby healthy tissues and/or organs (44). The high pressure of sound waves transmission, in conjunction with the intense duty cycle, result in an amount of energy that is absorbed by the irradiated tissue and transformed into heat, with local temperatures ranging from 60 ˚C to 85 ˚C (44). The elevated local temperature within the core of the affected malignant tissue induces coagulation and necrotic cell death (**figure 2**), whereas a milder thermal stress affects the surrounding tissue that borders the focal spot and results in apoptotic cell death (**figure 2**) (5). The presence of microbubbles, nanodroplets, or cavitation of small bubbles in fluids may enhance the FUS-induced local heating and cause mechanically derived injuries within treated malignant tissues (45-47). Thermal ablation can be conducted with high precision, since FUS-induced lesions size can be narrowed down to the range of a millimeter (48). The precision of the treatment can be further increased either with sonography, or magnetic resonance imaging (MRI) to guide and monitor the heating effects induced by the thermal ablation (49). The Food and Drug Administration (FDA) has approved thermal ablation for the treatment of uterine fibroids (50), prostate cancer (51), management of bone metastases-related pain (52) and essential tremor care for patients with Parkinson's disease, or fragile X-associated tremor/ataxia syndrome (53).

Thermal ablation affects the tumor microenvironment by inhibiting cell proliferation, degradation and/or denaturation of a variety of cellular factors and causing the swelling of endothelial cells, which results in reduced blood flow inside the tumor mass (54). Thermal ablation-induced occlusion of feeder vessels causes the loss of vascular elasticity, destruction of the capillary endothelium, cavitation of peritubular cells, incomplete plasma membrane and sever damage of the tumor capillary ultrastructure (34). The ensuing traumatic cancer cell death produces debris, along with the discharge of damage associated molecular patterns (DAMPs) and

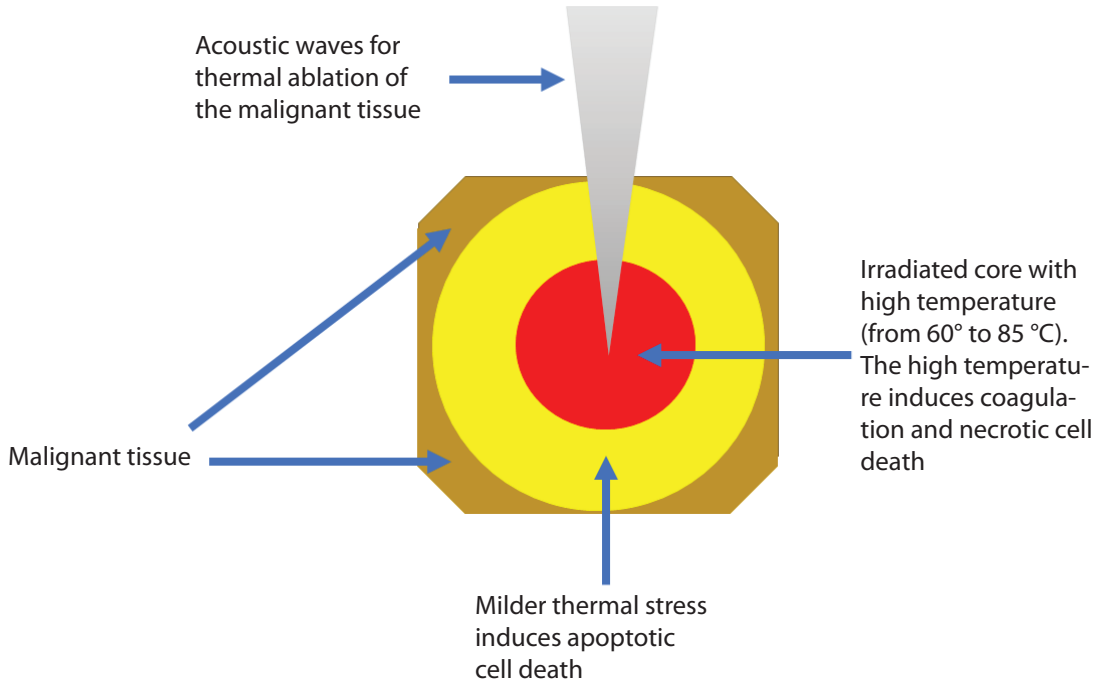

Figure 2. *Schematic representation of acoustic wave-induced thermal ablation of a tumor mass. The acoustic waves are denoted by the gray cone. The inner irradiated core with high temperature is reported in red, whereas the milder thermal stress is shown in yellow. The normal malignant tissue is represented in brown.*

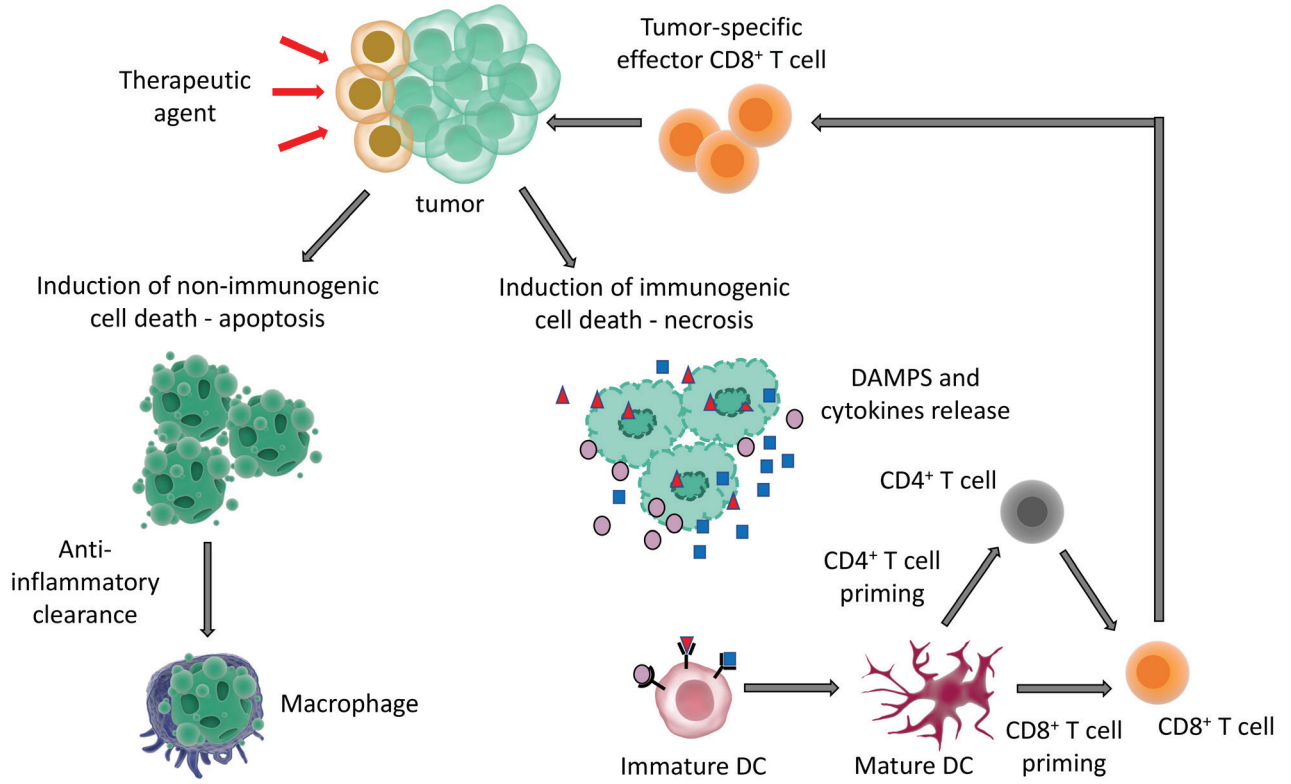

Figure 3. *Immunogenic and non-immunogenic cell death. A therapeutic agent destroys part of the malignant cells of the tumor mass. Some cells may undergo apoptosis, which does not elicit inflammatory reactions in the affected tissues, or organs. The cellular debris are subsequently removed by macrophages. Conversely, the necrotic or necro-apoptotic cell death releases cellular debris and other factors, which stimulate inflammatory reactions through DAMPs. The inflammation may ultimately lead to the maturation of dendritic cells, which then program tumor-specific cytotoxic CD8-positive T cells to attack the remaining cancer cells in the tumor mass and, possibly, in other regions of the organism (image taken from: Ventura E, Costa A, Dominguez RB, Romano G. Abscopal effects induced by localized interventions in oncological patients. Ann Res Oncol. 2021;1(4):272-84. doi: 10.48286/aro.2021.29).*

heat shock signals, which result in immunogenic cell death (16, 17) (**figure 3**). All the released inflammatory factors can be detected by surrounding antigen presenting cells, with consequent activation of host immune responses against tumor associated antigens (5, 16, 17, 55-60).

HYPERTHERMIA AND THERMAL STRESS

Both FUS-induced hyperthermia and thermal stress heat malignant tissues to milder temperatures compared to thermal ablation (61). Typically, areas treated with hyperthermia and thermal stress reach temperatures from 40 °C to 45 °C. The difference between hyperthermia and thermal stress depends on the time of the treatment. Hyperthermia requires a treatment period of 30 to 90 minutes, whereas thermal stress only requires a timeframe that ranges from seconds to a few minutes (61).

Hyperthermia and thermal stress have been applied in cancer care as adjuvants in order to elicit immune responses against the tumor, both in radiation therapy and chemotherapy and thermally activated drug delivery (61, 62).

Hyperthermia interferes with the cell cycle progression by targeting proteins and inducing DNA damage, which leads to traumatic cell death and/or apoptosis (63-65). Besides FUS, various techniques can be utilized to induce hyperthermia, such as: microwaves, radiofrequency and old-fashion physical methods based on warm water and heated air (66). In addition to inducing hyperthermia, FUS has the advantage of provoking mechanical damage in treated malignant tissues (67). At a cellular and molecular level, hyperthermia causes a short-term decrease in RNA synthesis and a longer-term decline in DNA synthesis in the S and M cell cycle phases, which result in a reversible cell proliferation arrest while some malignant cells undergo apoptosis (68). Moreover, hyperthermia sensitizes cancer cells to

radiation therapy and/or chemotherapy through the inhibition of the DNA repair pathways (69).

Hyperthermia induces vasodilation, which enhances blood flow and consequently causes a decline in hypoxia, interstitial tumor pressure and acidosis (67, 70). In summary, hyperthermia has a dual effect: it induces the death of malignant cells that releases DAMPs and increases the blood flow in the treated area, which signals cells of the immune system to enter the tumor mass. The combination of these two effects may activate the host immune system against cancer cells (16, 17).

Among the various systems for ultrasound-induced hyperthermia for clinical applications, the FDA has approved two devices named Sonotherm 1000 and Sonalleve (71, 72). Sonalleve is a commercially available magnetic resonance imaging system that guides hyperthermia-based treatments. FUS-induced hyperthermia has been utilized in clinical trials in combination with radiation therapy and/or chemotherapy for the treatment of a variety of malignancies, such as: ovarian cancer, metastatic pelvic tumors, prostate cancer, head and neck tumors, rectal cancer, glioma, hepatocellular carcinoma, and gastric cancer (73-81).

MECHANICAL STIMULATION WITHOUT THERMAL EFFECTS

The application of FUS to induce mechanical stimulation without thermal effects in irradiated tissues is also termed mechanical perturbation (82-87). The avoidance of thermal effects while imparting mechanical stimulation into cells and/or tissues can be achieved with low to moderate pressure combined with high-duty cycle (82-87). Sonoporation allows for transient apertures in cellular and/or tissues structures, such as cellular membranes and/or the three types of cellular junctions (86, 87). The focused ultrasound parameters for mechanical perturbation are adjusted to produce sonoporation-induced vasodilation, temporary opening of the blood-brain barrier and sonoporation of malignant tissues (88-92).

The effects of mechanical perturbation can be enhanced with microbubbles, by means of either acoustic pressure-induced fast expansion and contraction cycles, or cavitation (93). The rapid expansion and contraction of the microbubble is likely to cause a sudden rupture that may induce traumatic cell death by impairing the cellular membranes and affecting cellular metabolism (94, 95). In comparison, the biological effects of cavitation derive from the stable oscillation of the microbubbles, which creates a modest shear stress (93). The entity of the cellular membrane impairment may result either in apoptotic or necrotic cell death, and/or cell lysis (16, 17, 94, 95). Injured cells can repair minor cellular membrane damages and survive, or alternatively undergo apoptosis (94, 95). Conversely, extensive damage to the cellular membrane that cannot be repaired will only cause necrotic cell death (94, 95).

In preclinical studies, the increase of the vascular and cellular permeability induced by the combination of LOFU with microbubbles can be utilized to optimize the efficiency of nanoparticle carriers for drug and gene delivery (96). Following the interaction between the acoustic waves with the irradiated tissues in the presence of microbubbles, the acoustic radiation force (ARF) moves the oscillating microbubbles along the vascular system walls, enhancing the extent of the mechanical stress (97, 98).

MRI-guided sonoporation in combination with circulating microbubbles was applied in a phase 0 clinical trial for the temporary opening of the blood-brain barrier in patients with infiltrating gliomas (99). The transcranial administration of microbubble-enhanced sonoporation was safe and effective in causing the temporary opening of the blood-brain barrier (99). This finding is very promising for the development of novel and noninvasive interventions to treat patients with gliomas. For instance, the transient opening of the blood-brain barrier might optimize the efficiency of therapeutic agents in patients with brain tumors. Other studies have addressed the possibility of utilizing MRI-guided sonoporation for the transient opening of the blood-brain barrier (100, 101), which consequently may allow for the release of brain tumor markers into the bloodstream, such as circulating tumor DNA (100), or eventually, circulating tumor cells (101).

HISTOTRIPSY

Histotripsy is the most recent ultrasound-based technique for the modulation of host immune responses in cancer therapy (102, 103).

Histotripsy, also termed M-HIFU, or cavitation cloud histotripsy, requires very high pressure in combination with short-pulse ultrasound waves, which reduces the treated malignant tissue into an emulsion that is subsequently absorbed by the organism (102, 103). The pulse of these ultrasound waves is in the range of microseconds and pro-

duces condensate cavitating bubble clouds that undergo quick expansion and contraction (104), which, in turn, generates a mechanical force that smashes the affected tissue, with negligible thermal impairment (105).

A second type of histotripsy is termed boiling histotripsy. It requires longer pulses, which are still in the range of microseconds and produce boiling bubbles (106). The mechanical liquefaction of the treated soft tissue derives from the interaction between the boiling bubbles and the incident shockwaves (106). Boiling histotripsy fractionates the treated tissue into subcellular fragments without thermal impairment (107). By means of longer pulses, boiling histotripsy can also impart thermal impairment, in addition to the mechanical damage caused to the treated tissue (107).

Boiling histotripsy requires lower peak pressures than cavitation cloud histotripsy (106-108). For this reason, boiling histotripsy protocols can be more easily adapted to the needs of HIFU-based clinical applications (108). The consistency of the malignant tissue also plays a role in choosing the type of histotripsy for the treatment (109). For instance, tumor masses that have a high content of fibrotic tissues tend to be more resistant to histotripsy-based intervention (110). In these cases, cavitation cloud histotripsy might be more appropriate than boiling histotripsy for the treatment of the tumor.

Histotripsy-induced emulsification of tumor deposits is essentially mechanical, which may lead to the release of tumor-associated antigens that are not denatured by thermal effects and can stimulate an immune response (56, 110). Differences in eliciting host immune responses against a tumor between boiling histotripsy and thermal ablation based FUS were reported in a mouse EL4 thymoma model, using MRI and histopathology analyses (111). Boiling histotripsy-induced lesions exhibited forms of microhemorrhages on the border between the injured and unaffected tumoral tissue. The injured tumor mass contained a dense concentration of necrotic and apoptotic malignant cells and infiltration by macrophages and granulocytes was detected 4 days post-treatment (111). Instead, the thermal ablation-based FUS on the tumor exhibited the following effects: no sign of hemorrhage in the lesion produced by the treatment, heat-fixed malignant cells were observed in the central area of the treated tumor mass and macrophages and granulocytes were only present on the edges of the lesion (111). Preclinical studies are currently underway for the characterization of the effects of histotripsy on the vascular and immune systems in the context of different types of tumors (112-114).

Histotripsy was utilized for the first time in a clinical trial conducted in Barcelona, Spain for the treatment of 8 patients with either primary or multifocal liver metastases, derived from various kinds of primary tumors, such as colorectal cancer, hepatocellular carcinoma, breast cancer and gallbladder carcinoma (this information is available from: https://www.fusfoundation.org/the-technology/timeline-of-focused-ultrasound/first-histotripsy-clinical-trial/).

Histotripsy will be utilized in a clinical trial that is currently recruiting patients with primary or metastatic liver cancer (this information is available from: https://clinicaltrials.gov/ct2/show/NCT04572633). A similar approach will be adopted in another clinical trial for the treatment of patients with renal cancer (https://clinicaltrials.gov/ct2/show/NCT05432232).

CONCLUSIONS

FUS-based anticancer therapies are certainly safer than radiation therapy because acoustic waves do not induce the adverse effects that usually result from the use of ionizing radiations. Another main advantage is that FUS interventions do not require surgical procedures that necessitate hospitalization and, in turn, reduce the risk of infection.

Effective anticancer therapeutic approaches require long-term effects, in order to minimize the onset of metastases and/or the relapse of the disease in patients. In this regard, the FUS performance must be optimized for the treatment of oncological patients (1-15). To this end, preclinical studies are combining the effects of immune checkpoint inhibitors with various types of FUS-based treatments in attempt to produce long-term host immune responses against the tumor and potentially increase the occurrence of abscopal effects among oncological patients (16, 17, 115).

Another critical issue with FUS interventions is related to the lack of an appropriate characterization of the FUS-induced biological effects in oncological studies. For example, an interesting property of FUS applications in cancer therapy is associated with increased vascular permeability, which, on one hand, may optimize the delivery and efficacy of various types of anticancer agents, but, on the other hand, an increased vascular permeability might facilitate the dissemination of metastases in patients (116).

Lastly, additional studies must determine whether malignant cells may eventually become resistant to FUS-based treatments.

In summary, more efficient anticancer therapies may be achieved through a better understanding of FUS-associated biological effects in cancer therapy, along with the full characterization of the various combinations of the paraments that regulate and optimize the production of acoustic waves in FUS therapeutic applications in the field of oncology.

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Conflict of interests

The Authors have declared no conflict of interests.

Availability of data and materials

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Authors' contributions

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

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N/A.

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SYSTEMATIC REVIEW

RAPID SYSTEMATIC REVIEW OF CLINICAL TRIALS ON PHARMACOLOGICAL THERAPIES FOR RARE GYNECOLOGICAL CANCERS

Klejda **Harasani** [1], Neil T **Conlon** [2], Stephanie **Darmanin** [3], Aleksandar **Čelebić** [4], Milan Paul **Kubelac** [5], Amelja **Lala** [1], Jean **Calleja-Agius** [6, *]

[1] Faculty of Medicine, University of Medicine Tirana, Tirana, Albania
[2] National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland
[3] IQVIA, Stockholm, Sweden
[4] Clinical Center of Montenegro, Podgorica, Montenegro
[5] Oncology Institute Prof Dr Ion Chiricuta, Cluj Napoca, Romania
[6] Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Msida, Malta
[*] Correspondence to: jean.calleja-agius@um.edu.mt, https://orcid.org/0000-0001-6369-0297.

ABSTRACT: The purpose of this study is to systematically review clinical trials on pharmacological therapies for rare gynecological cancers and analyze their characteristics. The PRISMA guidelines for systematic reviews were followed and two databases were searched (WHO´s International Clinical Trials Registry Platform and clinicaltrials.gov). The Jadad score was used to assess the methodological quality of completed clinical trials. A total of 212 records, covering trials from 1993 to 2022, were included in the final review. More than half were phase II trials (110; 51.89%) and the status of recruiting was mainly completed (80; 37.74%). There were 26 (12.26%) terminated or withdrawn clinical trials. Just 42.45% of the trials were specific only for rare types of gynecological cancers. The most common type of investigated therapy was chemotherapy (89; 41.98%), followed by targeted therapy (64; 30.19%) and a combination of therapies (23.11%). However, in the last five years there was an increase in trials investigating targeted therapies such as immunotherapy, overgrowth-related and angiogenesis-related therapies. All completed trials except one, had a Jadad score 0-2, indicating low-quality. Thirty-six (45.00%) completed clinical trials had neither posted results, nor publications. Higher quality clinical trials with better reporting of results are needed for rare gynecological cancers

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Impact statement: Despite the increase over the years in the number of trials investigating pharmacological therapies (especially targeted therapies) for rare gynecological cancers, higher quality clinical trials and better reporting of results are needed.

Key words: *rare gynecological cancer; clinical trials; chemotherapy; targeted therapy; immunotherapy.*

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INTRODUCTION

Currently, it is estimated that about 50% of gynecological cancers are rare (annual incidence of less than 6 per 100.000) (1). Nevertheless, they account for more than 80,000 new cases per year in Europe. Based on the 2020 WHO classification of female genital tumors, gynecological rare cancers include more than 30 histologic entities, located in different sites (vulva-vagina, uterine cervix, uterine corpus, fallopian tube, ovary etc.) and of different morphology (epithelial, germ cell, sex cord stromal, trophoblastic, mixed *etc*.) (2). As with most rare diseases, these types of cancers share similar challenges: poor diagnosis, scarce research opportunities, insufficient expertise, few clinical trials, and limited treatment options (3). Initial treatment for almost all rare gynecological cancers involves surgery, however systemic treatment is also part of the standard of care, including hormonal therapy, chemotherapy, immunotherapy and targeted therapies (4).

Randomized clinical trials are considered as the gold standard in epidemiological research, however due to poor accrual rates and ethical reasons this study design is generally not applied to rare diseases (5).

Over the last decade, there has been a significant increase in biomarker-based studies, clinical trials for immuno-oncology treatments and targeted therapies (6). The development and clinical use of new drugs that target gene alterations regardless of the tumor´s site and morphology has led to a new era of precision cancer medicine. Clinical trials design has notably evolved, especially in the first phase. For example, rapid phase I dose-escalation clinical trials are succeeded by large expansion cohorts. Novel clinical trials with more complex design include adaptive platform trials, basket trials, and umbrella trials. Adaptive platform trials comprise planned opportunities to adjust or adapt specific elements of the design according to the analysis of data acquired from patients, thus increasing the trials´ efficiency (7). In an oncology basket trial, one targeted treatment is simultaneously evaluated for a variety of cancers that share a common genetic alteration or molecular defect (one targeted treatment in a basket with several types of cancers). In an umbrella trial, multiple targeted treatments in different treatment arms are evaluated for one type of cancer stratified by molecular defects or genetic alterations (an umbrella with several treatments assigned according to the molecular characteristics targeting one type of cancer) (8, 9). Among the factors associated with problematic clinical trials are poor study design, poor recruitment, ineffective site selection, patient burden, and poor trial execution (10). The integration of artificial intelligence and mobile health technology (such as wearables and sensors) has the potential to transform and optimize clinical trials, particularly in oncology (11). Patients´ participation in clinical trials and their overall engagement is crucial to speed up funding and support research on rare gynecological cancers. Initiatives including the European Network of Gynecological Cancer Advocacy Groups (ENGAGE) are being implemented to promote patients´ involvement and collaboration in research (12). However, there is an underrepresentation of specific population groups in oncology clinical trials in general, such as children, adolescents and young adults, adults lacking the capacity to consent, older adults, as well as racial and ethnic minorities (13-16). This is particularly important in the case of rare gynecological cancers, which are prevalent among girls and young women. Addressing the under-representation of such groups and promoting inclusion is important to have more accurate and representative outcomes and expand access to new cancer treatments. Moreover, one recent study found that patients with rare cancers are significantly less willing to participate in clinical trials compared to patients with common cancers (17).

Despite the challenges, clinical trials on common and rare gynecological cancers have advanced in investigating targeted therapies including antiangiogenic agents, PARP inhibitors, tumor-intrinsic signaling pathway inhibitors, selective estrogen receptor down-regulators, and immune checkpoint inhibitors. This has led to several drugs recently approved by the FDA for these malignancies, such as bevacizumab (target: VEGFR), olaparib, rucaparib and niraparib (target: PARP), pembrolizumab (target: Anti PD-1) and pembrolizumab + lenvatinib (targets: anti PD-1 + VEGFR) (18). Higher benefits and lower grade 5 risk seem to occur in phase I oncology clinical trials which target only one tumor type and incorporate biomarkers among the eligibility criteria (19).

The vast variability in the different types of rare gynecological cancers poses a challenge to fully comprehend the trends on therapies investigated as part of clinical trials for these particular malignancies. Therefore, it is of utmost importance to synthesize the evidence from clinical trials, elucidate their characteristics and explore the type of therapies involved. A multidisciplinary approach, international consortia and increased resources are indispensable to harmonize research, improve diagnosis, and design suitable clinical trials, thus leading to better treatments and patients´ outcomes (20). When searching the Cochrane library for previously published systematic reviews on therapies for rare gynecologic cancers, only one systematic review had been carried out on a specific type of rare gynecological cancer, namely malignant germ cell ovarian tumor. The authors reported that the small number of included studies (one RCT and one retrospective) and of patients (32 women) were insufficient to give conclusions on the effectiveness and safety of chemotherapy (21). Among the most active current international projects on the topic, the COST Action CA-18117 GYNOCARE has established a multidisciplinary European network for Gynecological Rare Cancer research: from concept to cure. Members from Working Group 5 "Coordination of interactions between clinical trials, translational research, and basic research" have focused on developing a clinical trial reference depository and systematically

reviewing clinical trials for rare gynecological malignancies (22). As members of this Working Group, the objective of our study is to comprehensively analyze the characteristics of clinical trials (finalized and ongoing) on pharmacological therapies for rare gynecological cancers.

MATERIALS AND METHODS

The PRISMA 2020 guidelines for systematic reviews and meta-analysis (23) were followed and two different databases were searched. The WHO´s International Clinical Trials Registry Platform (ICTRP) and the clinicaltrials.gov databases were consulted, as well as the articles published in PubMed/MEDLINE reporting on results of the included clinical trials on rare gynecological cancers. The WHO ICTRP is a platform that serves as a database collecting data from various clinical trial registries. Its mission is to enable complete access to research to everyone involved in health care decision making (24). Clinicaltrials.gov is the largest public registry of clinical trials offering information to diverse stakeholders (researchers, healthcare providers, patients, and their families) regarding ongoing and completed clinical trials (25). The protocol for the systematic review has been registered in PROSPERO.

A full list of search terms for each database is provided in **Appendix 1**.

Inclusion criteria included, regarding the type of study, only interventional, clinical trials that investigated pharmacological therapies for at least one rare gynecological cancer, from inception till June 2022, with primary purpose treatment. Exclusion criteria were: study designs different from clinical trials, observational studies and studies that did not include pharmacological therapies.

Rare gynecological cancer was defined based on the list of rare gynecological cancers as adapted from the WHO classification of tumors (26, 27).

Two researchers independently screened for clinical trials that reported on pharmacological therapies for at least one rare gynecological cancer (K.H. and A.L.). Screening of search results against eligibility criteria was performed from 07.26.2002 to 08.26.2022. Discrepancies were resolved by reassessment of the full record and discussion between reviewers (S.D., J.C.-A.).

The complete record of each clinical trial was reviewed and the following data were extracted: registration number (NCT), study name/official title, study type/design, funding/sponsor, research site/locations, research institute, stage, status, (estimated) start date, (estimated) completion date, population included, sample size (estimated), cancer/cancers type, recruitment period, intervention group measures, control group measures, outcome measures (primary and secondary), random methods, blind methods, distribution concealment, measurement indicators, results (published or not), publications.

The treatments in the clinical trials were classified according to the type of therapy: chemotherapy, hormonal therapy, targeted therapy, and mixed (more than one type of therapy). Drugs of targeted therapies were listed, classifying them into: 1) overgrowth-related (apoptosis induction and proliferative signaling), 2) angiogenesis-related, and 3) immunotherapy (28, 29).

A separate analysis was performed for completed clinical trials. The average length, type of therapies, drugs/treatments, and design characteristics were recorded. The Jadad score was used to assess the methodological quality of the clinical trials, with regards to masking, randomization, and accountability of all patients, including withdrawals. This validated score lies in the range 0-5, where scores of 3 or more indicate higher quality trials (30). Excel® (Microsoft, Redmond, Washington) was used for analysis of data. Results were presented as frequencies (%) or means with range and standard deviation (SD), as appropriate.

RESULTS

Overall, 779 records were identified in both databases (520 from clinicaltrials.gov and 259 from WHO ICTRP). After removing 85 duplications, 694 clinical trials were eligible for screening. A total of 482 records were excluded for the following reasons: not including rare gynecological cancers (327), studies on prevention or quality of life (61), medical procedures such as radiotherapy or diagnostic procedures (55), observational studies (24), and preventive vaccines (15). Finally, a total of 212 records, covering trials recorded during the period 1993 - 2022, were included in the final review.

Figure 1 shows the PRISMA 2020 flow diagram for the systematic review. **Supplementary material 1** provides the full list of the included clinical trials.

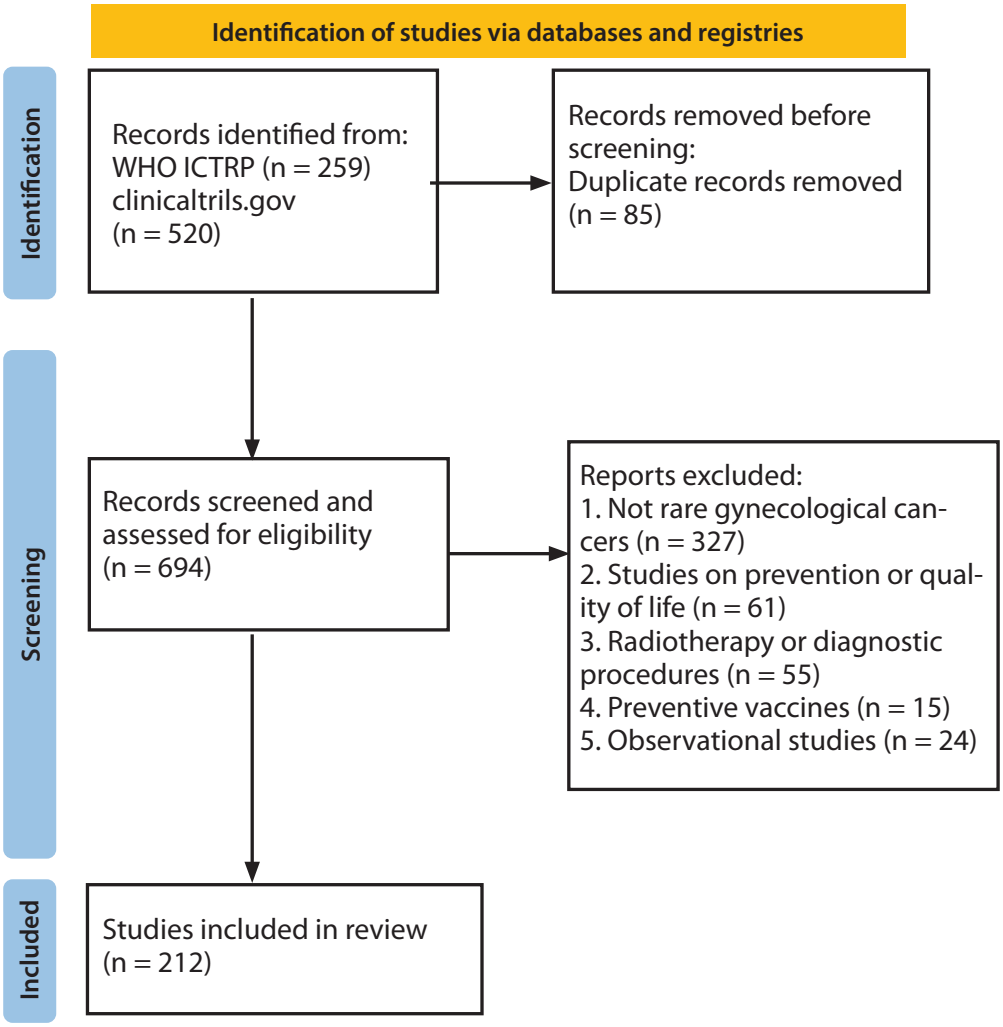

Figure 1. *PRISMA 2020 flow diagram for the systematic review.*

CHARACTERISTICS OF CLINICAL TRIALS

These interventional clinical trials included in the 212 records recruited, or estimated to recruit, a total of 30941 patients. Median number of patients included in the trials was 40, ranging from 0 (in seven withdrawn clinical trials) to 2059 (SD 299.73). Five clinical trials did not provide the number of patients. Most of the clinical trials included only female patients (145; 68.40% of the trials). This is because there were clinical trials which included men with testicular tumors, and other related tumors.Adult and older adult patients were eligible to be included in 99.06% and 95.75 % of the trials (210 and 203), respectively. There was a significantly lower number of clinical trials that included children (13.68%; 29 clinical trials).

Table I summarizes the characteristics of clinical trials for therapies on rare gynecological cancers included in our study.

More than half of the trials were phase II trials (110; 51.89%), followed by phase I (53; 25.00%), and phase III (32; 15.09%); the rest of them were phase I/II, phase II/III, phase III/IV and three reported not applicable (N/A). The status of recruiting was mainly completed (80; 37.74%), recruiting (59; 27.83%), and active, not recruiting (25; 11.79%). There were 26 (12.26%) terminated or withdrawn clinical trials due to various reasons such as low accrual (reported in 8 clinical trials), lack of approval by the ethics committee, sponsor decision to withdraw funding, principal investigator departure from institution or due to toxicity. Funding was public in 101 clinical trials (47.64%), private in 66 (31.13%), private, non-profit in 40 (18.87%), and the remaining (5; 2.36%) were mixed, unclear or not provided. Among the most frequent sponsors were: The National Cancer Institute (31) (45; 21.23%), universities or university hospitals (36; 16.98%) and the Gyne-

Table I. *Characteristics of clinical trials on pharmacological therapies for rare gynecological cancers.*

CLINICAL TRIAL CHARACTERISTIC	TYPE	NUMBER (%)
Status	Completed	80 (37.74)
	Recruiting	59 (27.83)
	Active, not recruiting	25 (11.79)
	Terminated or withdrawn	26 (12.26)
	Other (not yet recruiting, ongoing, suspended, unknown)	22 (10.38)
Phase	I	53 (25.00)
	I/II	11 (5.19)
	II	110 (51.89)
	III	32 (15.09)
	Other (II/III/IV/IV, not applicable	6 (2.83)
Funding	Public Private Private, non-profit Other (mixed, unclear, not provided)	101 (47.64) 66 (31.13) 40 (18.87) 5 (2.36)
Primary outcome measure	Efficacy Safety Both efficacy and safety Not provided	108 (50.94) 41 (19.34) 47 (22.17) 16 (7.55)
Therapy	Chemotherapy Hormonal therapy Targeted therapy Mixed Other	89 (41.98) 6 (2.83) 64 (30.19) 49 (23.11) 4 (1.89)

cologic Oncology Group (GOG foundation) (32) (25; 11.79%). The trials were mainly conducted in the USA: 142 or 66.98% of the trials, followed by Canada: 25 or 11.79%. One of the trials was carried out in Mexico. Study locations in Europe were distinctively fewer and placed mostly in the United Kingdom and France (both 15; 7.08%), followed by Italy (14), Germany (11), and Austria (6). Most of the clinical trials were international and multicenter, being carried out in multiple different countries.

Related to the type of cancer, 90 (42.45%) clinical trials including only patients with rare gynecological cancers were carried out, 67 (31.60%) clinical trials including patients with gynecological cancers encompassing at least one rare type, and 55 (25.94%) clinical trials including patients with various types of cancers, among them rare gynecological cancers. A vast heterogeneity was noted on the subtype of rare gynecological cancers included in clinical trials, comprising almost all sites, morphology, and types of malignancy. Some of the most prevalent were adenocarcinomas of the cervix, ovary, fallopian tube, and vulva-vagina, ovarian and uterine germ cell tumors, leiomyosarcomas, uterine carcinosarcomas, and uterine adenosarcomas.

We identified one basket trial, a multi-center phase II trial of nivolumab and ipilumab as dual anti-PD-1 and anti-CTLA-4 blockade in rare tumors (33).

Primary endpoints (outcome measures) of the clinical trials related largely to efficacy (50.94%), safety (19.34%) or both efficacy and safety (22.17%). Among the efficacy endpoints were: progression free survival (PFS), overall survival, objective response rate (ORR), and event-free survival (EFS). Safety endpoints were: frequency and severity of adverse events, dose-limiting toxicities (DLT), maximum tolerated dose (MDT).

PHARMACOLOGICAL THERAPIES INVESTIGATED IN THE INCLUDED CLINICAL TRIALS

The most common type of therapy investigated in clinical trials for rare gynecological cancers was chemotherapy (89; 41.98%), followed by targeted therapy (64; 30.19%), and mixed therapies (49; 23.11%), generally a combination of chemo-

therapy and targeted therapy. Hormonal therapy was rarely investigated (6; 2.83%), similarly to other therapies (4; 1.89%) such as propranolol, radiation modifier triapine and VCN-01 virus.

Table II and **table III** provide a complete overview of the therapies that were investigated in clinical trials on rare gynecological cancers (RGC) which were included in this review, classified by the type of therapy and group of drugs.

TRENDS OF CLINICAL TRIALS ON PHARMACOLOGICAL THERAPIES FOR RARE GYNECOLOGICAL CANCERS OVER TIME

The number of clinical trials investigating therapies for rare gynecological cancers (RGC) has steadily increased over the years, reaching 79 clinical trials initiated in the period from 2017 to 2022. **Figure 2** shows the number of clinical trials for these malignancies stratified by the type of therapy over the years. Moreover, the most evident finding is the increment in trials involving targeted therapies. Indeed, 35 new clinical trials on targeted therapies started in the last five years compared to just 26 from 2000 to 2016. Furthermore, during the last five years (2017-2022) there has been an increase in clinical trials investigating a combination of therapies (targeted therapies plus chemotherapy) and a decrease in clinical trials focused on chemotherapeutic agents only.

ANALYSIS OF COMPLETED CLINICAL TRIALS

A separate analysis was performed for the 80 completed clinical trials which were eligible for inclusion in this systematic review. The average length for these clinical trials was 59.77 months, median 53 months (SD 35.322). Indeed, the shortest trial lasted 9 months (34), investigating sunitinib in treating patients with recurrent or persistent leiomyosarcoma of the uterus, while the longest period was 17 years and 11 months (35), investigating combination chemotherapy plus peripheral stem cell transplantation in relapsed germ cell cancer.

Overall, 54 different drugs or treatments were included in the completed clinical trials. The type of therapy for rare gynecological cancers in these trials was mostly chemotherapy (43;

Table II. *Classification of chemotherapy and hormonal therapies in clinical trials on RGC.*

TYPE OF THERAPY/GROUP OF DRUGS	DRUGS, TREATMENTS
Chemotherapy	
Alkylating agents	
Nitrogen mustards	busulfan, melphalan
Platinum based	cisplatin, carboplatin, oxaliplatin, nedaplatin
Oxazaphosphorines	cyclophosphamide, ifosfamide, palifosfamide
Tetrazines	temozolomide
-	trabectedin, lurbinectedin
Purine analogs	dacarbazine
Antimetabolites	
Purine antagonists	fludarabine
Pyrimidine antagonists	5-fluorouracil, capecitabine, gemcitabine
Antifolates	pemetrexed
Mitotic spindle poisons	
Vinca alkaloids	vinorelbine
Taxans	paclitaxel, nab-paclitaxel, docetaxel
Microtubule inhibitors	ixabepilone, eribulin
Others	
Antibiotics	bleomycin, mitomycin C
Proteasome inhibitors	bortezomib
Topoisomerase I inhibitors	irinotecan, topotecan
Topoisomerase II inhibitors	etoposide, doxorubicin
Hormonal therapy	
Estrogen receptor antagonists	tamoxifen, fulvestrant
Progestin	levonorgestrel
Aromatase inhibitors	letrozole
Somatostatin analog therapies	177Lu-DOTATOC (177Lu-octreotate/octreotide)

Table III. *Classification of targeted therapies in clinical trials on RGC.*

TYPE OF THERAPY / GROUP OF DRUGS	DRUGS, TREATMENTS
Targeted therapies	
Overgrowth related targeted therapies (apoptosis induction and proliferative signaling)	
HER2 inhibitors	trastuzumab
mTOR inhibitors	everolimus, temsirolimus
PARP inhibitors	olaparib, rucaparib, niraparib, veliparib
MEK inhibitors	MEK162 or binimetinib, selumetinib, trametinib
MEK/RAF inhibitors	VS-6766
AKT inhibitors	ipatasertib
AKT/ERK Inhibitors	ONC201
ATR kinase inhibitors	AZD6738 or ceralasertib
Glutaminase inhibitors	IPN60090
BMI1 inhibitors	unesbulin
CDK4/6 inhibitors	palbociclib, ribociclib
CDK9 inhibitors	alvocidib
G2 checkpoint kinase (WEE1) inhibitors	ZN-c3
IDO1 inhibitor	epacadostat
FRα-binding antibody	mirvetuximab, STRO-002
HMT Inhibitor, EZH2-Inhibitor	tazemetostat
Gamma-secretase inhibitors	RO4929097
PDGFR-α inhibitor	olaratumab
Folate receptor targeted therapies	Folic-acid functionalized C'Dot-Drug-Conjugate (FA-CDC) ELU001
Angiogenesis related targeted therapies	
VEGF inhibitors	bevacizumab, sunitinib, cediranib
EGFR inhibitor	nimotuzumab, panitumumab, cetuximab
Tyrosine kinase inhibitors	imatinib, nintedanib, dasatinib, cabozantinib, tivozanib, brivanib, lenvatinib, vadalanib, pazopanib, anlotinib
Aurora A kinase inhibitors	alisertib
FAK inhibitors	defactinib
Immunotherapy	
Immune checkpoint inhibitors	
Inhibitor of CTLA-4	ipilimumab, tremelimumab, XmAb®22841 or bavunalimab
Inhibitor of PD-1	nivolumab, pembrolizumab, dostarlimab, PF-06801591 or sasanlimab
Inhibitors of both CTLA-4 and PD-1	XmAb20717 or vudalimab, lorigerlimab
Inhibitor of PD-L1	durvalumab, atezolizumab, avelumab
Inhibitor of TIGIT	etigilimab
Chimeric (human-mouse) monoclonal antibody	dinutuximab
Murine monoclonal antibody	oregovomab
Claudin 6-targeted half-life extended bispecific T-cell engager (HLE BITE®)	AMG794
CD47 blocker	TTI-622
Biological	Recombinant interleukin-12, GEN-1 IL 12
Tumor vaccines	pNGVL3-hICD vaccine pUMVC3-hIGFBP-2 multi-epitope plasmid DNA vaccine Attenuated Live Listeria Encoding HPV 16 E7 Vaccine ADXS11-001 DC-006 vaccine Personalized vaccine peptides
CAR T-cell therapy	huCART-meso Cells CAR.B7-H3 LCAR-M23 cells

HER2: human epidermal growth factor receptor 2; mTOR: mammalian target of rapamycin; PARP: poly ADP-ribose polymerases; MEK: mitogen-activated protein kinase; CDK: cyclin dependent kinases; FRα: Folate receptor alpha; HMT: Histone Methyltransferase; PDGFR-α: platelet-derived growth factor receptor alpha; VEGF: Vascular endothelial growth factor; EGFR: epidermal growth factor receptor; FAK: focal adhesion kinase; CTLA-4: cytotoxic T-lymphocyte associated protein 4; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1; TIGIT: T cell immunoglobulin and ITIM domain; CAR: Chimeric antigen receptor.

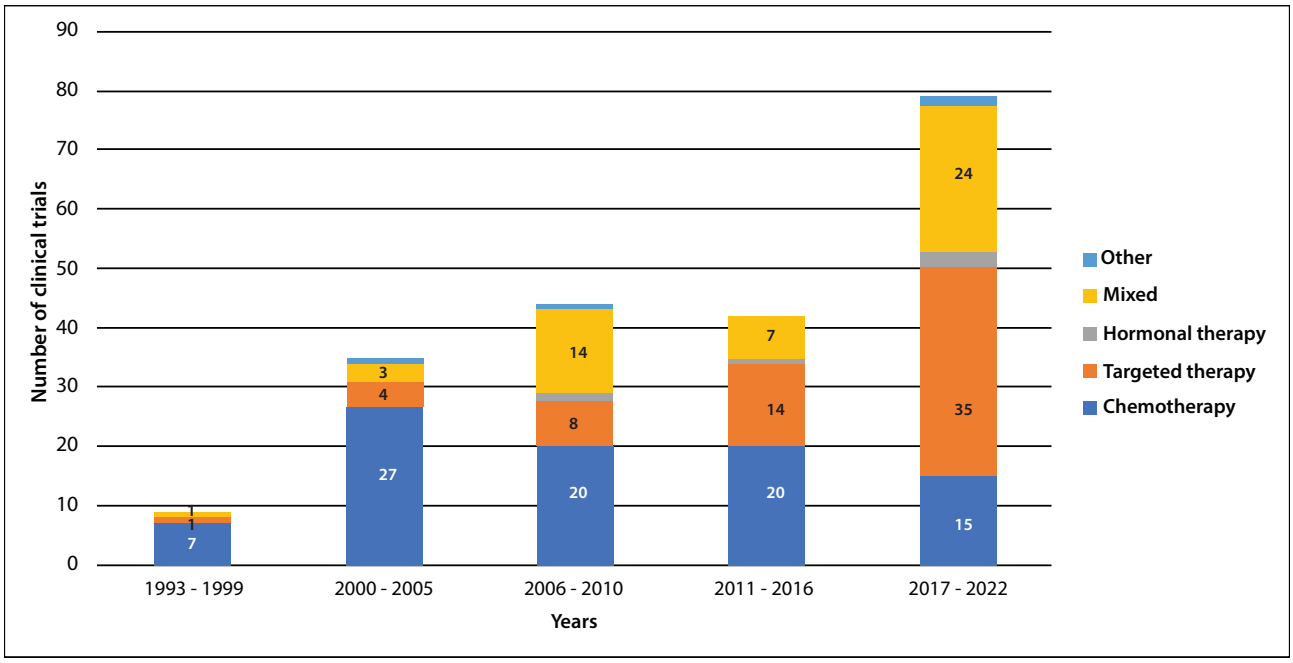

Figure 2. *Number of clinical trials on rare gynecological cancers and type of therapy over the years.*

53.75%), targeted therapy (19; 23.75%) and a combination of therapies (16; 20%). Out of 80 completed trials there was a predominance of phase II (47), phase I (20) and less often phase III (9). More than half (43; 53.75%) had public funding. The average number of patients enrolled in completed phase I clinical trials was 36.68 (ranging from a minimum of 11 to a maximum of 90 patients), for phase II clinical trials 131.35 (range 10-1873), and for phase III this figure was 304.89 (range 28-1520).

Regarding the study design, allocation was not applicable (49, 61.25%), non-randomized (10; 4.72%) and randomized (10; 4.72%), while for the remaining trials the information was not provided. Intervention model was mostly single group assignment (53; 66.25%), parallel assignment (8; 3.77%) or not provided (15; 7.08%). Masking was double (participant and investigator) in just two clinical trials, being the majority of clinical trials open label (69; 86.25%).

All completed trials except one (36), had a calculated Jadad score from 0 to two, indicating low-quality trials. Only 28 (35.00%) of the completed trials had posted results in the searched databases, and 30 (37.50%) had publications, either in databases or identified in Medline with the National Clinical Trial (NCT) number. Thirty-six (45.00%) completed clinical trials had neither posted results, nor publications.

DISCUSSION

This rapid systematic review provides valuable insight on the landscape and characteristics of clinical trials that investigate pharmacological therapies for patients with rare gynecological cancers. Just 42.45% of the 212 clinical trials were specific only for rare types of gynecological cancers. This implies that treatments for this group of cancers are more often studied together with other types of tumors. Phase II clinical trials predominated (51.89%), being more than all other phases together. We identified only one basket trial in our study. Basket trials enroll patients with the same genetic driver mutations, regardless of the tissue or organ of origin, including all types of cancer, both rare and common ones (37).

A considerable number of clinical trials for these rare malignancies (26; 12.26%) was withdrawn or terminated for various reasons, where the most common were related to slow accrual of patients, sponsor decisions, and limited funding. In the present review, funding was public in 47.64% of the trials. Despite hurdles concerning design and implementation, there is a growing need for first-in-human clinical trials for patients with rare gynecological cancers. These are imperative in the era of precision medicine to investigate appropriate novel treatments.

Closer cooperation between public and private institutions, and patients' organizations seems the best approach (38).

Our review revealed that overall, the majority of clinical trials included predominantly chemotherapy (41.98%), followed by targeted therapy (30.19%) and a combination of therapies (23.11%). In line with this finding, in a recent review on clinical trials for drug development in rare diseases, the authors found out that a small number of rare diseases (mostly cancer-related) contributed to the majority of trials and investigated drugs (39), hence, chemotherapeutic drugs were the most abundant in the top 20 tested drugs. In contrast, a study by Chen *et al.* on therapies for prostate cancer in China found that clinical trials were focused mainly on hormonal therapy (41.5%), chemotherapy (31.3%) and immunotherapy (20.7%) (40). This may be explained by the different types of treatment options due to different pathogenesis for these distinct cancers, which is reflected in the different profile of investigated therapies within clinical trials.

Not surprisingly, an evident increase was observed over the years in the total number of clinical trials investigating therapies for rare gynecologic cancers, particularly of those including targeted therapies such as immunotherapy (immune checkpoint inhibitors, tumor vaccines, CAR-T cells), overgrowth-related and angiogenesis-related targeted therapies. Indeed, 54 clinical trials investigating chemotherapy for these malignancies were initiated from 1993 to 2010, compared to 35 in the period from 2011 to 2022. Quite the opposite was observed for targeted therapy; 13 clinical trials from 1993 to 2010, compared to 49 that were initiated in the period from 2011 to 2022. This trend follows the same direction as in treatment regimens of other types of cancer, explained by the introduction of novel therapies with new mechanisms of actions. According to a report published by IQVIA, a paradigm shift in oncology drug expenditure from chemotherapy and hormonal therapy to targeted approaches has occurred over the past 25 years (41).

The heterogeneity of the subtype of rare gynecological cancer does make patient recruitment more challenging. The fact that these rare gynecological cancers can affect girls, adolescents and women of childbearing age is an added burden when planning these clinical trials and seeking ethics approval (42).

There was only little variation in the design of completed clinical trials, where most were open-label (no masking) with single group assignment and allocation was not applicable as they were single-arm trials. Only one completed clinical trial had a calculated Jadad score higher than 2 (Jadad score =3) which indicates higher quality. This phase III clinical trial of carboplatin and paclitaxel with or without bevacizumab in women with stage III or IV epithelial ovarian, primary peritoneal or Fallopian tube cancer was randomized, double blind and included a description of withdrawals and dropouts. (36). Results were unpublished in 65% of the completed clinical trials and 45% of them had neither publications, nor had posted any results. These findings highlight the need for higher-quality clinical trials and better reporting of data from the current ones.

LIMITATIONS OF THE STUDY

We did not include trials that investigated radiotherapy, surgery, or other procedures, as this particular review was focused on clinical trials comprising at least one pharmacological therapy. These could be studied in future systematic reviews to further shed light on existing literature. Our study may underestimate the number of basket trials, as they include many different types of cancer which exhibit the same biomarker or mutation, without specifying the site, morphology or histologic diagnosis. Furthermore, the majority of clinical trials lacked published results and/or publications, making it difficult to extract such information.

Clinical trials in rare gynecological cancers are a main research priority. "Smarter" and higher quality clinical trials are needed with the involvement of multiple centers in different countries. Patient engagement through advocacy groups is key in order to enhance education and awareness on current research. Examples include the European Network of Gynecological Cancer Advocacy Groups (ENGAGe) (43). More awareness about the importance of biobanking, particularly in the case of rare gynecological cancers, will hopefully increase patient participation, and thus lead to the design of better multicenter clinical trials. Establishing international networks focusing on rare gynecological research, from basic research to cure, such as GYNOCARE (44) needs to continue to be encouraged in order to reach out to the

key stakeholders. This will bring together experts in basic science research, pathology, oncology, surgery, gynecology, pharmacy, and other specialties together with lawyers, ethicists and data protection officers in order to help bridge the gap with pharmaceutical companies and industry, and lead to effective well-designed clinical trials which will start to address the lacunae in our current knowledge of how to best manage patients with rare gynecological cancers.

CONCLUSIONS

This systematic review gives a snapshot of clinical trials on pharmacological therapies for rare gynecologic cancers and their characteristics.

The paucity of high-quality trials limits evidence-based decisions in clinical care, therefore it is essential to design and implement novel trials with good quality and better reporting of results.

COMPLIANCE WITH ETHICAL STANDARDS

Fundings

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Conflicts of interests

The Authors have declared no conflict of interests.

Availability of data and materials

The data presented in this study are available in the **Supplementary Material 1** and Annex A. Further data is available on request from the Corresponding Author.

Code availability

N/A.

Authors' contributions

KH and JCA: conceptualization; KH: writing; KH, NC, SD, AC, MK, AL and JCA: writing-review and editing; JCA: funding acquisition. All Authors have read and agreed to the published version of the manuscript.

Ethical approval

Human studies and subjects

Since this study involved the review of exisiting published research, no new patients or study participants were recruited. Hence no ethical approval was needed.

Animal studies

Since this study involved the review of exisiting published research, no new animal studies were carried out. Hence no ethical approval was needed.

Publication ethics

Plagiarism

This is a review article and all original studies are cited as appropriate.

Data falsification and fabrication

N/A.

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APPENDIX 1

The complete list of search terms for each of the databases

1.1 Search terms in www.clinicaltrials.gov

gynecological tumors, rare
rare gynecological cancers, neuroendocrine
malignant female reproductive system neoplasm, rare
gynecological tumors, neuroendocrine
gynecological cancer, vaginal, vulvar
uterine adenosarcoma
rare gynecological cancer, ovarian cancer
ovarian clear cell carcinoma
germ cell tumors, gynecologic
ovarian sertoli-leydig cell tumor
ovarian germ cell tumors, neoplasm, cancer, malignancy
dysgerminoma
PARP-inhibitors, CD-4 inhibitors, MEK inhibitors, anti HER2

1.2 Search terms in WHO ICTRP https://www.who.int/clinical-trials-registry-platform

rare gynecological cancer
rare gynaecological cancer
rare gynecologic cancer
"gynecological cancer" and rare
rare diseases and orphan drugs "gynecological cancer"
"epithelial ovarian cancer" rare diseases and orphan drugs
low grade serous carcinoma
leiomyosarcoma
ovarian cancer, epithelial
vulvar cancer
vaginal cancer
uterine cervix cancer

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CASE REPORT

"A SHEEP IN A WOLF'S CLOTHING" - CASE REPORT OF TUBERCULAR AXILLARY LYMPH NODES MIMICKING METASTASIS IN A CASE OF BREAST CARCINOMA WITH BRIEF REVIEW OF LITERATURE

T. K **Aravind** [1,*], N **Yeswanth** [2], Zeya **Monirah** [3], Antony **Sebu** [4]

[1] Department of Urology, VMMC & Safdarjung Hospital, Delhi, India
[2] Department of Surgical Oncology, Sri Venkateshwara Institute of Medical Sciences, Andhra Pradesh, India
[3] Department of Surgery, UCMS & GTB Hospital, Delhi, India
[4] Department of Surgery, Delhi State Cancer Institute, Delhi, India
[*] Correspondence to: aravindtk2793@gmail.com, https://orcid.org/0000-0002-6501-5014.

ABSTRACT: "Appearances can be deceptive" – the saying holds true in our case where sinister appearing enlarged axillary lymph nodes draining breast carcinoma were surprisingly found to be tubercular. A 60-year-old nulliparous female presented with complaints of a right breast lump with no comorbidities. Examination and evaluation revealed a retro areolar non - metastatic breast carcinoma of clinical stage (cT2N1M0). Surprisingly, the operative specimen revealed all isolated lymph nodes to be free of tumor but with features of granulomatous lymphadenitis raising a suspicion of tuberculosis which was confirmed by further tests. On the basis of Multi-disciplinary Board (MDB) discussions and literature research the patient was started on anti-tuberculous treatment (Category-1) with adjuvant chemotherapy planned after the intensive phase. Problems of diagnosis and management arise when breast carcinoma and tuberculosis coexist in unique scenarios. Hence it would be worthwhile for the reading surgeon to keep an eagle eye on the lookout for such cases as wrong treatment strategies in such scenarios could prove disastrous. Major lacunae do exist in many aspects of managing such cases with larger studies being the way forward helping formation of standard guidelines to approach such complex encounters. We portray this case with a brief summary of similar published scarce literature and discussion on the various aspects of management of such coexistences based on the available literature evidence.

ABBREVIATIONS: MDB: Multi-disciplinary Board; TB: Tuberculosis; IDC: Invasive Ductal Carcinoma; FDG: Fluorodeoxyglucose.

Doi: 10.48286/aro.2022.58

Impact statement: Established protocols of evaluation and management exist already in the various aspects of managing common disorders like breast carcinoma and tuberculosis but management aspects become complex when the disorders co-exist as naturally no fixed protocols exist in approaching these cases considering extreme rarity. This article describes this rare and unique case scenario with a summary of published scarce literature thus far with thought provoking new insights on the subject.

Key words: *breast carcinoma, tuberculosis, anti-tubercular treatment, case report, oncology.*

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INTRODUCTION

The famous Greek philosopher Anaxagoras once said "Appearances are a glimpse of the unseen" portraying the fact that external appearances seldom depict internal dangers. This saying holds true in coexistence of breast carcinoma and tuberculosis where in our case sinister appearing FDG avid axillary lymph nodes draining breast carcinoma, thought to be metastatic were surprisingly found to be tubercular altering management decisions. Literature search revealed less than 30 such cases reported till date deeming this coexistence to be distinctly rare (1-4). We present a case of invasive ductal carcinoma of the breast coexisting with ipsilateral tubercular axillary lymph nodal enlargement mas-

querading malignant infiltration with a brief discussion on the aspects of difficulties faced during evaluation and management of such cases with a brief review of scarcely available literature on the subject.

PATIENT INFORMATION

60-year-old nulliparous female presented with complaints of a right breast lump. She had no comorbidities nor any history of relevance per-

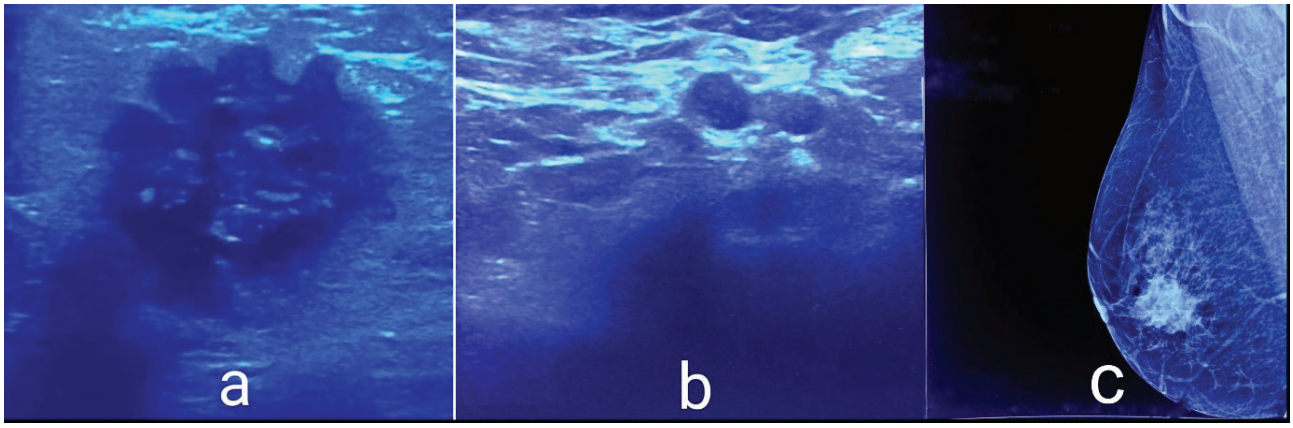

Figure 1. *Local Imaging.* ***a****. USG Breast showing well defined Irregular Hypoechoic lesion in the right breast with lobulated/angular margins and rich vascularity likely malignancy;* ***b****. USG Axilla showing Enlarged Hypoechoic lymph node in the axilla with loss of central hilum likely metastatic;* ***c****. Mammography of the right breast showing a BIRADS 5 retro areolar lesion.*

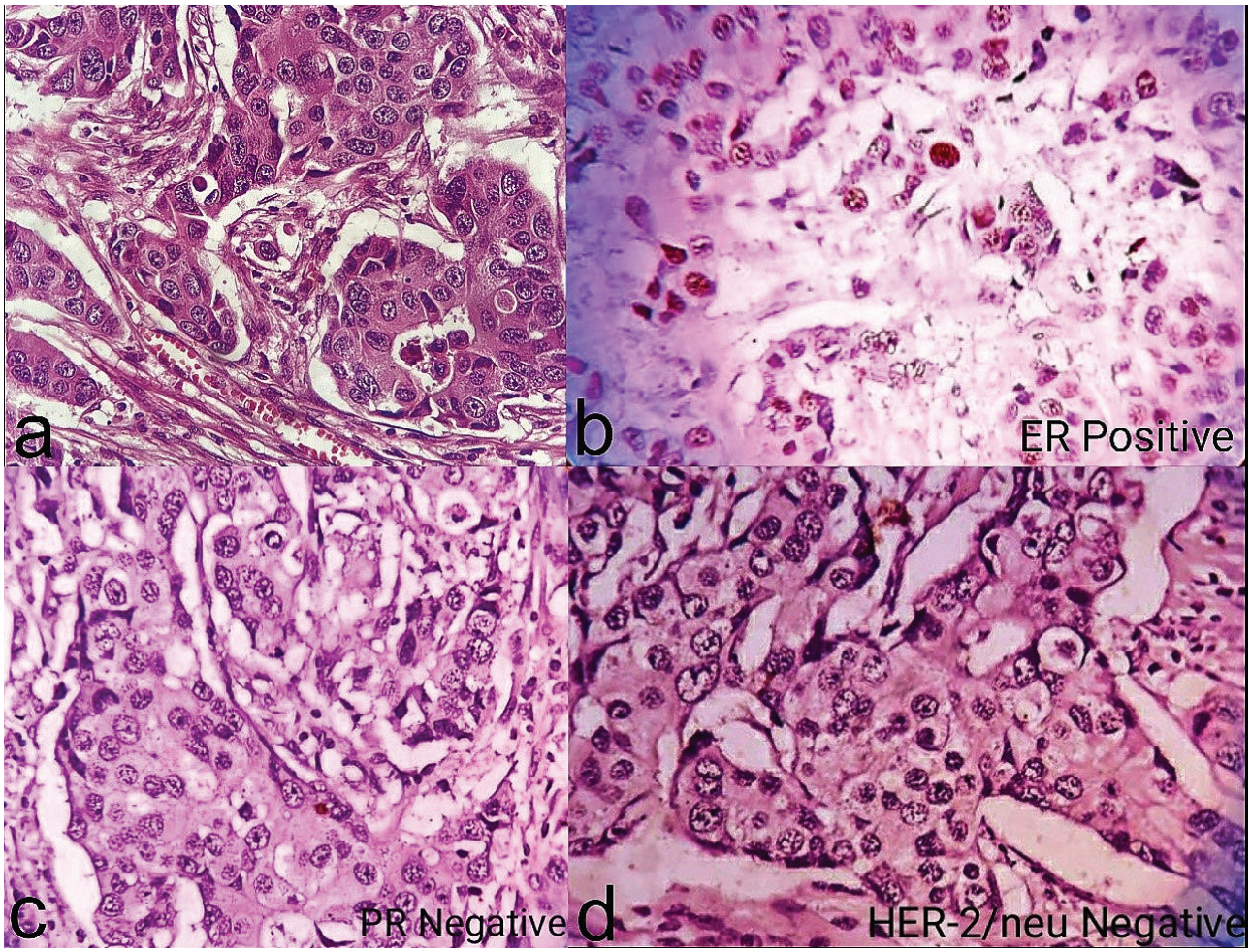

Figure 2. *Pathological evaluation of the right breast lesion.* ***a****. 40x magnification revealing invasive ductal carcinoma, no special type (NST);* ***b-d****. IHC images showing Luminal A status.*

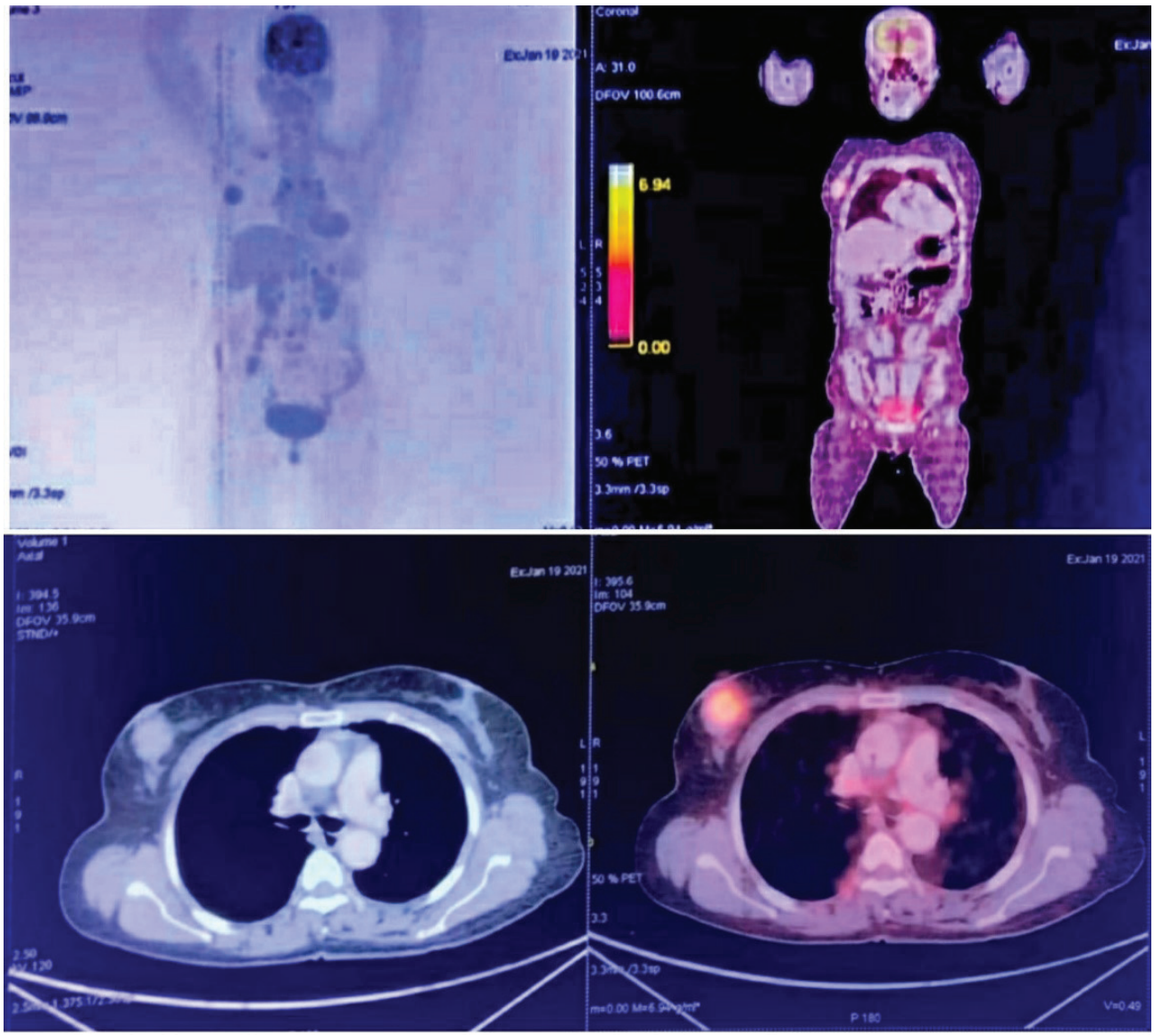

Figure 3. *18F-FDG PET-CT scan showing FDG avid heterogeneously enhancing central right breast mass involving the nipple areola complex with underlying pectoralis being free of tumor. Multiple enlarged FDG avid axillary lymph nodes in levels 1 to 3 largest measuring 2 x 1 cm.*

taining to the lump except a recently diagnosed hepatitis B infection for which she was started on Entecavir. Examination revealed a retro areolar lump of 4 × 3.5 × 3.5 cm dimensions and ipsilateral multiple mobile axillary lymphadenopathy with mammography and ultrasound axilla confirming the same **figure 1**.

Histopathology revealed Invasive Ductal Carcinoma (IDC) – No Special Type (NST) with Luminal A status **figure 2**. The patient presented to us with an 18F-FDG PET scan (**figure 3**) suggested to her by her referring physician that showed a FDG avid central right breast mass with multiple enlarged ipsilateral axillary lymph nodes with malignant characteristics reconfirming our clinical staging ($cT_2N_1M_0$). The disease was staged early breast carcinoma and a Modified Radical Mastectomy (MRM) was performed which revealed the same histology.

Surprisingly, all 26 lymph nodes isolated from the specimen were free of tumor but had features of *granulomatous lymphadenitis* raising a suspicion of tuberculosis (TB). Further Ziehl Neelsen (ZN) staining of the lymph nodes revealed acid fast *bacillus* compatible with TB **figure 4**. Discussion of this unique case was done in a Multi-disciplinary Board meeting (MDB) and with expert opinions from oncologists and literature research, the patient was started on anti-tuberculous treatment (Category 1) with adjuvant chemotherapy planned after the intensive phase.

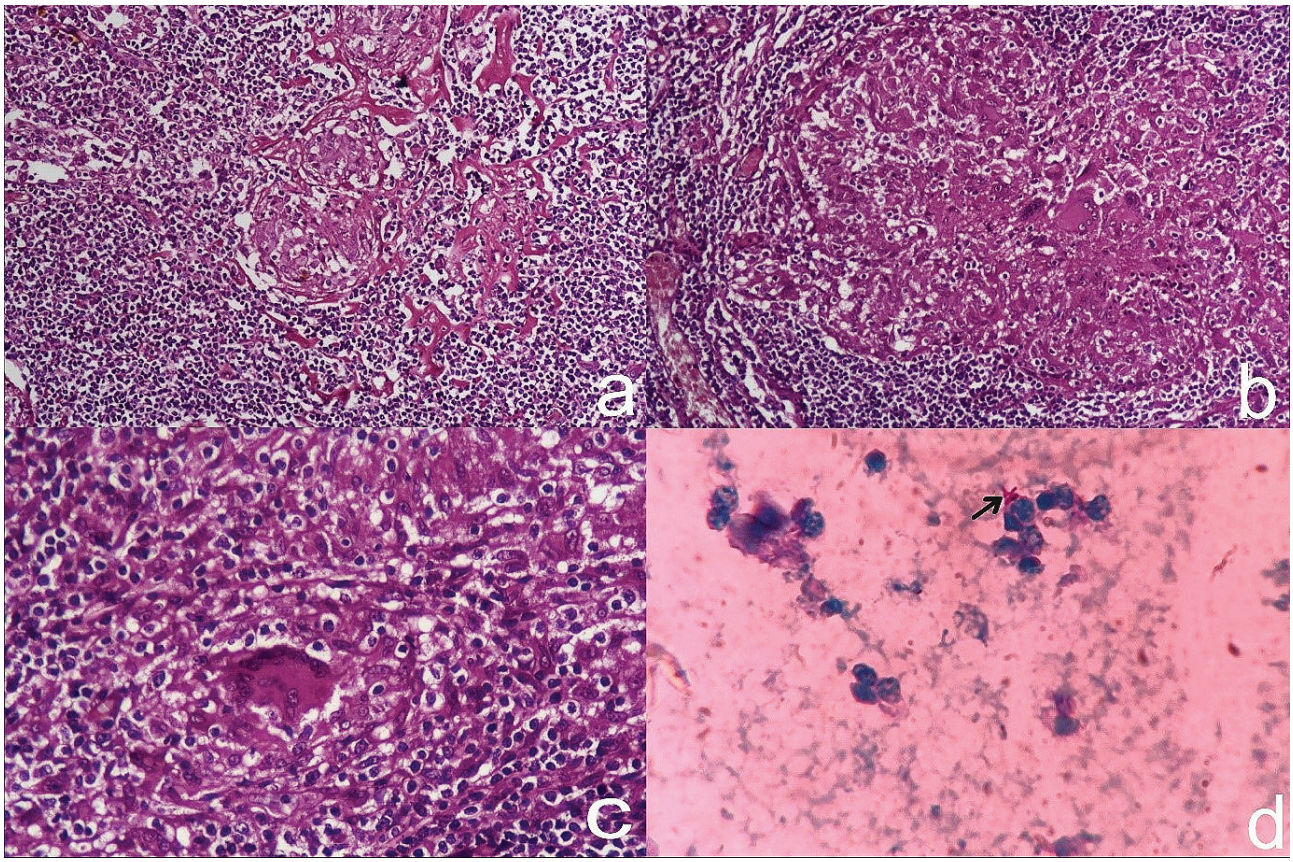

Figure 4. *Pathological evaluation of the right axilla.* ***a****. H&E staining showing granuloma with surrounding Lymph node parenchyma (10x Magnification);* ***b****. H&E staining showing granuloma with a giant cell (10x Magnification);* ***c****. H&E staining showing Langerhans giant cell in great detail (40x Magnification); d. Ziehl Neelsen staining showing acid fast bacilli suggestive of M. Tuberculosis (Arrowed marked).*

DISCUSSION

Despite the high prevalence of breast carcinoma and axillary tuberculosis in Asia, coexistence of both has rarely been reported in literature (1-4). As in most reported cases, the diagnosis of axillary tuberculosis in our case was also incidental and post-operative as there were no systemic evidence to provoke any suspicion towards tuberculosis during preoperative evaluation nor is the coexistence of TB with IDC common. Nevertheless, establishing a preoperative diagnosis of axillary tuberculosis in such cases does carry certain advantages, but diagnosing/suspecting coexistence preoperatively is difficult with the current diagnostic pathways utilised in breast carcinoma. The notable advantages of a preoperative diagnosis would include avoidance of a possible over staging of disease status leading to a change in both prognosis and treatment protocols (5, 6). Secondly of note other advantages would include avoidance of chronic wound complications associated with TB by early initiation of ATT prior to surgery. Finally, another major challenge in such cases would be problem of drug interactions between chemotherapeutic agents and anti-tubercular drugs (7) necessitating personalised therapeutic regimens as presently, no established guidelines exist to define treatment strategies in such unique situations. Literature search about similar rare case reports from the Asian subcontinent (**table I**) revealed certain common points noticed in most reports (8-18). Almost all reports excepting one (18) made the diagnosis post-operatively with individualised/personalised treatment protocols initiated on patient basis. ATT duration ranged from 6 months to 18 months with some authors preferring concurrent RT/CT (8, 9) while another school of thought of providing delayed adjuvant RT/CT after wound healing (11-17) also is being practised. Hence, on reviewing available literature, lacunae do exist in several aspects of management starting from diagnosing preoperatively to framing management protocols post diagnosis. This case reports also serves to add to the currently available scarce literature on the subject and also alerts the

Table I. *Review of similar Asian case reports on coexisting breast carcinoma and TB axilla.*

AUTHORS	AGE	LATERALITY	TB FOCI	MANAGEMENT STRATEGIES
Tulasi *et al*. (8) (Report of 5 cases) (2002)	36	Left	I/L Axilla	Simultaneous Adjuvant ATT & CT.
	49	Left	I/L Axilla	Simultaneous Adjuvant ATT & CT/RT.
	60	Right	I/L Axilla	Simultaneous Adjuvant ATT & CT/RT.
	60	Left	I/L Axilla	Simultaneous Adjuvant ATT & ET.
	81	Left	I/L Axilla	Simultaneous Adjuvant ATT & ET.
Vishnu *et al.* (9) (2020)	35	Left	I/L Axilla	Simultaneous Adjuvant ATT & CT followed by delayed CT.
Tallari *et al.* (10) (2016)	53	Left	I/L Axilla	Immediate Post-Operative ATT initiation.
Pujani *et al*. (11) (2015)	45	Right	I/L Axilla	Immediate Post-Operative ATT followed by CT/RT after wound healing.
Maharia *et al*. (12) (2015)	35	Right	I/L Axilla	Immediate Post-Operative ATT followed by CT/RT after wound healing.
Jagtap *et al*. (13) (2018)	50	Right	I/L Axilla	Immediate Post-Operative ATT followed by CT after wound healing.
Hariharan *et al*. (14) (2017)	71	Left	I/L Axilla	Immediate Post-Operative ATT followed by CT after wound healing.
Goyal *et al*. (15) (2013)	57	Left	I/L Axilla	Immediate Post-Operative ATT followed by CT/RT after wound healing.
Pandey *et al*. (16) (2003)	52	Left	I/L Axilla	Immediate Post-Operative ATT followed by CT/RT after wound healing.
Pandey et al. (17) (2014)	50	Left	I/L Axilla	Immediate Post-Operative ATT followed by CT after wound healing.
Mukhopadhyay *et al*. (18) (2015)	55	Right	C/L Axilla, Level 5 neck & Supraclavicular Regions.	Preoperative ATT Initiated followed Surgery and adjuvant RT.

TB: Tuberculosis; I/L: Ipsilateral; C/L: Contralateral; ATT: Anti-Tuberculosis Therapy; CT: Chemotherapy; RT: Radiotherapy.

reading surgeon to remain watchful in all cases of breast carcinoma and look out for such presentations as the axillary lymph nodal status in a histopathology report is often overlooked or ignored once the diagnosis of IDC is made.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interests

The Authors have declared no conflict of interests.

Availability of data and materials

All supporting data and materials elucidated in the manuscript is available with the Corresponding Author and the same would be shared for review upon request to the Corresponding Author.

Authors' contributions

TKA and NY were the responsible surgeons' in-charge of the overall care of the patient and on whom the final decision on the patient management rested. TKA and ZM wrote the first draft of the manuscript.

TKA, YN and AS were involved in day to day patient care and follow up.

ZM and AS provided insight and valuable inputs to the manuscript, collected references and were responsible for typography of final manuscript draft.

Ethical approval

Human studies and subjects

The informed consent has been obtained from the patient.

Animal studies

N/A.

Publication ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

N/A.

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CASE REPORT

MALIGNANT PROLIFERATING TRICHILEMMAL TUMOR: A RARE NEOPLASM IN A RARER LOCATION. CASE REPORT AND REVIEW OF THE LITERATURE

Marco **Montella** [1], Andrea **Farabini** [2], Stefano **Lucà** [1], Michele **Donati** [3], Alfonso **Baldi** [4, *]

[1] Pathology Unit, Department of Mental Health, Physic and Preventive Medicine, Luigi Vanvitelli University of Campania, Naples, Italy
[2] Istituto di Medicina e Scienza dello Sport Antonio Venerando, Rome, Italy
[3] Department of Pathology, University Hospital Campus Bio-Medico, Rome, Italy
[4] Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, Luigi Vanvitelli, University of Campania, Caserta, Italy
[*] Correspondence to: alfonsobaldi@tiscali.it, https://orcid.org/0000-0002-8693-3842.

ABSTRACT: Malignant proliferating trichilemmal tumor (MPTT) is an uncommon and malignant tumor of the skin, arising from the outer root sheath of the hair follicle. Most MPTTs occur in the head and neck area and affect the scalp predominantly. However, rarely, MPTT of other anatomical sites, such as back, buttocks, chest, elbow, vulva, mons pubis and nose, have been described. Although the malignant biological behaviour of the neoplasm, the known loco-regional aggression and the potential local and distant recurrences, surgical excision with free margins of normal tissue is generally curative and represents the gold standard of treatment. We describe a case of MPTT arising on the scrotum of 51-year-old man. Through this case and the review of the literature we try to establish the state of the art regarding the clinical aspects, the morphological and immunohistochemical features of this neoplasm. Finally, we underline the exceptionally rare location of the tumor in our case. To the best of our knowledge this is the first case of MPTT arising on scrotum.

Doi: 10.48286/aro.2022.55

Impact statement: Diagnosis and treatment of malignant trichilemmal tumor needs a multidisciplinary approach with a strong clinical-morphological correlation for an accurate diagnosis, particularly when it occurs in exceptionally rare sites.

Key words: *malignant trichilemmal tumor; trichilemmal malignant tumor; malignant proliferating trichilemmal tumor; proliferating trichilemmal carcinoma; MPTT.*

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INTRODUCTION

Malignant proliferating trichilemmal tumor (MPTT) is a rare, malignant skin tumor arising from the outer root sheath of the hair follicle (1). The term MPTT was originally employed by Saida *et al*. (2) in order to define skin neoplasms, diagnosed as Proliferating Trichilemmal Tumor (PTT), which showed malignant histological features as infiltrative growth pattern, nuclear atypia, high mitotic activity including atypical forms, and lymph node metastases (3). Even PTT is an uncommon skin tumor of the outer root sheath of the hair follicle with a benign but locally aggressive clinical behaviour (4). MPTT is considered to develop *de novo* or originate from a pre-existing trichilemmal cyst (TC) or PTT, which is known as proliferating trichilemmal cyst (5, 6). Most MPTTs arise on the scalp but they were also observed on the back, buttocks, chest, elbow, vulva, mons pubis and nose (7). Clinically, the tumor occurs in both males and females, but affects women more frequently (8) and it appears as a dermic or subcutis nodule with an exophytic or ulcerated appearance and it varies in size, from less than 1 cm to more than 10 cm (9). Histologically, MPTT shows marked cytological atypia, cellular pleomorphism, atypical mitoses, dyskeratotic cells, and infiltrative growth pattern (1, 4, 10). The prognosis of MPTT is variable, including local

aggression, local and distant recurrence potential, mainly depending on the treatment executed (5). The gold standard for treatment for MPTT is a wide local excision with a 1-cm margin of normal tissue, although some Authors have been used chemotherapy and radiotherapy to prevent recurrence (11).
We present a case of an unusual site of occurrence of MPTT with review of the literature.

MATERIALS AND METHODS

Our literature review was performed using the search medical databases PubMed, Scopus, Web of Science, and Google Scholar. Searches were performed using key terms "trichilemmal malignant", "trichilemmal malignant tumor," "trichilemmal malignant", "trichilemmal malignant tumor" and "malignant proliferating trichilemmal tumor", "proliferating trichilemmal carcinoma".

CASE REPORT

A 51-year-old man presented with a gradually increasing, painless nodular swelling over scrotum, since several months, which ulcerated with bloody discharge. There was no history of trauma or skin cancers in the past. On examination, there was a 1.5 × 2 cm fungating growth over the scrotal region. There was no evidence of regional lymph node enlargement, even at US analysis. An excisional biopsy was done and the diagnosis of MPTT was given. A wider local excision of the scar was performed later. Histopathological examination of the biopsy was performed as previously described (12, 13) and showed a skin covered irregular lobulated mass measuring 2 cm in maximum diameter with just focal surface ulceration (**figure 1**). Microscopically, the dermis showed a cellular tumor composed of lobules and sheets of squamous cells with abrupt keratinization (**figure 1**). The cells were highly pleomorphic with hyperchromatic nuclei and high mitotic activity with abnormal mitotic figure as shown in (**figure 2**). Multinucleated tumor giant cells and foci of invasion into surrounding tissue were evident. The diagnosis of MPTT was made. The resected margins were free of tumor. The histopathological examination of the wider local excision specimen showed a sclerotic scar without residual images referable to MPTT. After 6 months of follow-up there are no signs of local recurrence or disease progression.

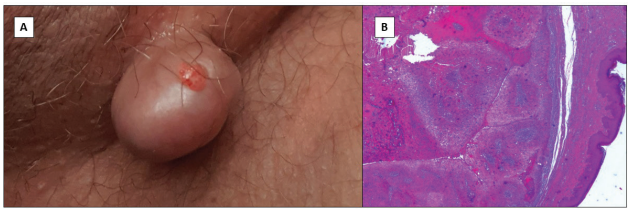

Figure 1. ***A.*** *Mobile, polypoid and ulcerated mass in the left region of the scrotum;* ***B.*** *Histologically the neoplasm shows a dermic localization, with a lobulated architecture and abrupt keratinization (4x magnification, H&E staining).*

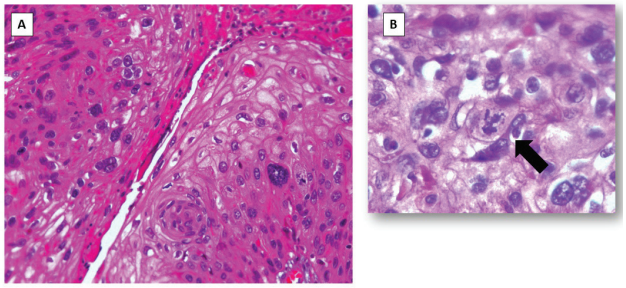

Figure 2. ***A****. High power magnification of MPTT. Atypical squamous cells with trichilemmal differentiation and evident nuclear pleomorphism;* ***B****. In a background of atypical squamous cells, atypical mitosis (black arrow) can be seen. (40x magnification, H&E staining).*

DISCUSSION

Proliferating trichilemmal tumors (PTTs) were originally described by Jones in 1966 as benign but locally aggressive skin neoplasms arising from the outer root sheath of hair follicles (14). MPTTs constitute less than 0.1% of skin cancers (15). The tumor usually appears on the scalp (>90% of cases), mostly affects women, occurring between the fourth and the eighth decade of life (16). Other rare site reported are the breast (17) arm (18), neck (19) jaw (20) sinuses (21) lip (22) eyelid (23) groin (24) orbit (25) and ear (26). Up to our knowledge this is the first case reported on the scrotum. The aetiology of this neoplasm is unknown, still, many of them originate from a pre-existing trichilemmal cyst (27). The progression of the tumor is slow but locally invasive. Ulcers may appear over time (28) as our case demonstrated. Histologic features of a MPTT that differentiate it from a benign TC or PTT include abnormal mitoses, high mitotic rate, marked cellular pleomorphism, architectural atypia, infiltrating margins, necrosis, and aneuploidy (8). The histopathological main differential diagnosis includes

ruptured trichilemmal cyst, giant trichilemmal horn and squamous cell carcinoma (SCC). Ruptured trichilemmal cysts may show irregular aggregates of keratinocytes with trichilemmal keratinization, but they should lack the solid multilobular structure and the cytological atypia. Giant cutaneous trichilemmal horns consist of a mixture of squamous epithelial cells and trichilemmal keratinized debris, mitoses can be common but not in the atypical form (29). PTTs are often confused histologically with metastatic or invasive SCC, as both can display keratinocytic nuclear atypia and infiltrative growth (30). On pathology, MPTTs are distinguished from SCC by trichilemmal keratinization involving an abrupt transition of nucleated to anucleated keratinized epithelium without granular layer. Sometimes can be found even invasive growth extending beyond the confines of the cyst wall, with irregular border, that make the differential diagnosis quite challenging (31). Immunohistochemical stains may be helpful tool. Herrero *et al*. reported that in the diagnosis of MPTT, cellular aneuploidy and CD34 loss are important markers (32). Moreover, p53 and Ki-67 markers in immunohistochemical examination are guiding in predicting the aggressiveness of the tumor (low grade-high grade). Decreased expression of p53, which is responsible for the repair of DNA damage, and increased expression of Ki-67, which indicates a high rate of mitosis in the cell, have been associated with malignant transformation and high recurrence rates (33-35). In a review of the literature, 93 well-documented cases of MPTTs were found, beyond our case (36-41). Furthermore, differential diagnosis between MPTTs and other skin cancer with squamous differentiation, including both squamous cell carcinoma and adnexal neoplasms with squamous differentiation can be challenging (42). We report a series of twenty-five MPTT (**table I**), using the search medical databases PubMed, Scopus, Web of Science, and Google Scholar Searches. The majority of cases occurred in female patients (15/25, 60%) and in the head and neck area (21/25, 84%), especially on the scalp (12/21, 57%). Radical surgical excision of neoplasm, with free-margins, was often curative, with no disease recurrence (15/21, 71.4%), and metastatic or relapsing disease was infrequently observed (6/21, 28%), with two (2/21.9%) cases of death for disease. In our knowledge this is the first case of MPTT arising on scrotum. MPTT was treated with surgical excision and resected margins were free of tumor. The outcome is satisfying, and the six-month follow-up showed no recurrence of disease. A guideline with a high level of evidence could not be established for their treatment since MPTTs are seen very rarely. According to most published paper surgery is favoured as gold standard, and the exact approach should be based on clinical judgment (35). Usually, surgical excision with a margin of 1 cm is the main step in the treatment of almost all MPTTs. Ye *et al.* (36) examined 76 proliferating trichilemmal tumors (PTT) in order to outline histopathologic features that could predict clinical behaviour and prognosis. Three distinct PTT variants were described based on malignant features such as nuclear atypical, mitotic activity, and presence of necrosis. Group I lesions are considered benign with recurrence not observed. Histopathologic examination reveals trichilemmal keratinization, focal nuclear atypia, mononuclear infiltrates, and absence of mitotic figures, perineural invasion, or vascular invasion. Group II lesions are low-grade malignant tumors with an increased risk of local recurrence. Histologically, these tumors are noted to have abrupt keratinization, areas of single-cell necrosis, and desmoplastic stroma. Cytologic atypia is absent. Group III lesions are high-grade malignant tumors with a high risk of recurrence, lymph node involvement, and/or distant metastasis. The case presented displayed histological features corresponding to group II lesion. Fortunately, our patient underwent surgery in the initial phase of the disease, therefore at the follow-up six months after the enlargement of the previous excision, there were no signs of disease, and no further therapy was necessary. Radiotherapy and chemotherapy have been used as adjuvant therapy in aggressive cases or recurrence (16). Patients should be followed closely and examined often to frequently assess recurrence or metastasis. Lymph node examination, US, CT, and in selected cases PET scan are recommended for pre-treatment staging. It is difficult to make accurate predictions about the clinical course since most of the MPTT cases in the literature have been reported as small case series.

CONCLUSIONS

MPTTs are rare adnexal tumors that require a multidisciplinary approach to diagnosis and treatment. Sometimes the morphological features are not easy to correlate with its clinical presentation, and diagnosis can be tricky, especially in unusual site. To create treatment algorithms, there is a need for increased case reports and comparative long-term results of different treatment options.

Table I. *Review of twenty-five MPTTs.*

N. CASES	AGE	SEX	SITE	ONSET	TREATMENT	OUTCOME	REFERENCE
1	Unknown	Unknown	Unknown	PTT with malignant transformation	SE	Unknown	*Markal et al.* (1)
2	66 Yr	Male	Scalp	De novo	SE	MD	*Ye et al.* (36)
	66 Yr	Female	Forehead	De novo	SE	MD	
1	63 Yr	Male	Scalp	De novo	SE	NR	*Tikku et al.* (30)
1	58 Yr	Female	Scalp	PTT with malignant transformation	SE	MD and DFD	*Mori et al.* (37)
3	64 Yr	Female	Scalp	De novo	SE	NR	*Herrero et al.* (32)
	53 Yr	Male	Scalp	De novo	SE	NR	
	66 Yr	Female	Scalp	De novo	SE	LTF	
1	52 Yr	Female	Scalp	De novo	SE	NR	*Alici et al.*
1	97 Yr	Female	Scalp	PTT with malignant transformation	SE	NR	*Fernando et al.* (8)
1	86 Yr	Male	Jaw	De novo	SE	Unknown	*Fernandez* (18)
1	58 Yr	Female	Scalp	NA	CT and RT	NR	*Casas et al.* (9)
1	65 Yr	Female	Scalp	De novo	SE	NR	*Gulati et al.* (39)
1	62 Yr	Female	Breast	De novo	SE	NR	*Akrami et al.* (40)
1	67 Yr	Female	Breast-Axilla	De novo	SE	NR	*Uchida et al.* (15)
1	63 Yr	Male	Arm-Wrist-Hand	De novo	SE	NR	*Aneiros-Fernandez et al.* (16)
1	32 Yr	Male	Neck	De novo	SE	LTF	*Durairaj et al.* (17)
1	56 Yr	Female	Jaw	NA	SE and CT	MD and DFD	*Hayashi et al.* (41)
1	53 Yr	Male	Sinuses	De novo	SE	ROD	*Harris et al.* (21)
1	75 Yr	Male	Lip	PTT with malignant transformation	SE	NR	*Kim HJ et al.* (22)
1	42 Yr	Female	Eyelid	De novo	SE	NR	*Lee et al.* (23)
1	80 Yr	Female	Orbit	De novo	Recommended RT	MD	*Ogul et al.* (25)
1	79 Yr	Male	Ear	PTT with malignant transformation	SE	NR	*Vandeweyer et al.* (26)
2	69 Yr	Female	Scalp	De novo	SE and RT	NR	*Cavanagh et al.* (5)
	53 Yr	Female	Scalp	De novo	SE	NR	

Yr: years; MD: metastic disease (lymph node and/or distant metastatis); SE: Surgical excision; NR: no recurrence; DOD: died for disease; LTF:lost to follow-up; CT: Chemotherapy; RT: radiotherapy; ROD: recurrence of disease; NA: not available.

COMPLIANCE WITH ETHICAL STANDARDS

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Availability of data and materials
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Authors' contributions
All the Authors listed in the manuscript contributed substantially to the conception and design of the work, or to the acquisition, analysis or interpretation of data for the work. All Authors drafted the work or reviewed it critically. All Authors have given final approval of the version to be published. All Authors agree to be responsible for all aspects of the work ensuring that issues relating to the accuracy or integrity of any part of the work are properly investigated and resolved.

Ethical approval
Human studies and subjects

The written informed consent was obtained from the patient before enrolment in the study to permit the use of the data generated in retrospective analyses.

Animal studies

N/A.

Publication ethics
Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

N/A.

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BRIEF REPORT

THE PROFESSIONAL USE OF SOCIAL MEDIA AMONG RESEARCH PROFESSIONALS IN THE MEDICAL FIELD. A NATIONAL MULTIDISCIPLINARY SURVEY

Sara **Testoni** [1, †], Rosita **Cenna** [2], Francesca **Mannozzi** [1], Veronica **Franchina** [3], Celeste **Cagnazzo** [2, *, †]

[1] IRCCS Istituto Scientifico Romagnolo per lo Studio dei Tumori (IRST) Dino Amadori, Meldola, Forlì-Cesena, Italy
[2] AOU Città della Salute e della Scienza di Torino, Ospedale Infantile Regina Margherita, Torino, Italy[3] A.O. Papardo Messina, Messina, Italy
[†] Equal contribution
[*] Correspondence to: celeste.cagnazzo@unito.it, https://orcid.org/0000-0003-4055-3139x.

ABSTRACT: Many benefits have been recognized concerning the use of social media in the medical field, especially in oncology and clinical research and can be an opportunity for a better relationship between patients and healthcare professionals. The scope of this project is to investigate the use of social networks for professional purposes among professionals working in clinical research in Italy. A specific anonymous survey was created by the Italian Group of Study Coordinators to explore the use of social media among clinical research professionals. The survey was composed of 13 questions grouped into different investigational sections and the attitudes and perceptions of professionals were assessed using ten-point Likert scales, continuous percentage scale or different answer options. According to respondents the most used social network for business purposes is Facebook (74.7%), followed by LinkedIn (69.0%), with Research Gate particularly appreciated in the 30-49 years range. The evaluation of the respondents with respect to the real usefulness of social networks for work is average (median score 5.93 on a 1-10 scale), without major differences between different age groups (5.90 in 18-29 years' group; 5.93 in 30-49; 5.66 in 50-55). The evaluation of the usefulness by cancer patients is also average (median score: 6.00), with a pessimism that characterizes above all the group of 50-65 years' respondents (5.18 *vs*. 5.86 in 18-29 years' and 6 in 30-49 years' group). Social media can help break down traditional barriers that prevent interaction among healthcare professionals as well as between providers, scientists, patients, and caregivers. Given the popularity and almost universal appeal of social media, we encourage physicians and institutions to learn and engage more in this ongoing evolution.

Doi: 10.48286/aro.2022.59

Impact statement: The use of social media by Italian clinical research professionals is quite widespread but characterized by a profound heterogeneity among professional figures and in the different hospitals.

Key words: *social media; clinical research; clinical research professionals; patients; social networks.*

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BACKGROUND

In the last few years, the use of social media has expanded in different fields. Since their creation, social networks have spread with an extraordinary speed and have become a widespread and permanent phenomenon in our society. By establishing social relationship through the network, users give way to a different type of social structure, which in one way or another influences the people involved (1).
Availability and preferences of different social networks vary across countries. Facebook is currently the world's largest social network, with more than 2.01 billion users worldwide. Twitter, with more than 330 million of monthly active users, has become essential to scientific conferences, providing publicity via sharing real-time proceedings or live-tweeting. Social network sites provide platforms for users to share their own content, react, or add comments on the content posted by other users. They help strangers to connect based on their common interests, activities, identities, or professions. LinkedIn, with more than 530 million members in over 200 countries and territories, focuses on business connections and industry contacts for employers and working professionals. It

allows users to enhance their connections in their areas of expertise. WhatsApp Messenger provides free of charge, cross-platform communication to more than 1 billion people in over 180 countries (2). Shared networking attracts different users and allows them to communicate based on their needs and interests (3). Because we use in multiple social networks and occupy different social positions in different settings, the relationship between social networks and health encompasses everything from the flow of viruses and information to the sharing of emotions, opinions, behaviors, and resources, all of which may spread in different ways and through different parts of our social networks (4). Over the decades, these connections and interactions through social networks have transferred face-to-face encounters to cyberspace. However, just like the introduction of the telephone, internet communication has not replaced face-to-face contact but has complemented it and personal networks are no longer restricted by geography and physical space (5).

Social networks are widely used in health communication and research and provide platforms to the public to access health information and to seek support when needed. A new dimension to healthcare was created to enable the public, patients, and healthcare professionals to discuss on health issues and facilitate the improvement of health outcomes. In a meta-analysis, social network sites' interventions were found to be effective in changing health behavior–related outcomes in which the predominant health domain was fitness related (*e.g.*, weight loss and physical activity). Emerging evidence supports the use of social network sites among health professionals to develop virtual communities for sharing domain knowledge (2).

The use of social media in medicine has increasingly recognized benefits including its use in oncology and clinical research (6). In oncology practice, the use of social networks can be an opportunity for both patients and healthcare professionals to improve their relationship (7). Therefore, we can see how the approach to clinical research has deeply evolved with positive aspects, particularly ethical and confidentiality aspects (8).

There are many authors who have reported how a correct professional use of social platforms may offer countless advantages in healthcare: facilitating interactions between patients, physicians, and the academic community (9), leading to an enhancement in clinical trial recruitment (10), cancer screening and early diagnosis (11); creating a hub where patients and staff can listen, learn, engage, and co-create to advance cancer care (12). Furthermore, several professionals claim that the social media virtual dimension offers several opportunities for patient education, research dissemination, as well as professional development for health care providers (13).

In the clinical research field, social media is used principally to communicate in order to connect with the public, attract and recruit patients (10), and disseminate clinical trial information (14). **Figure 1**

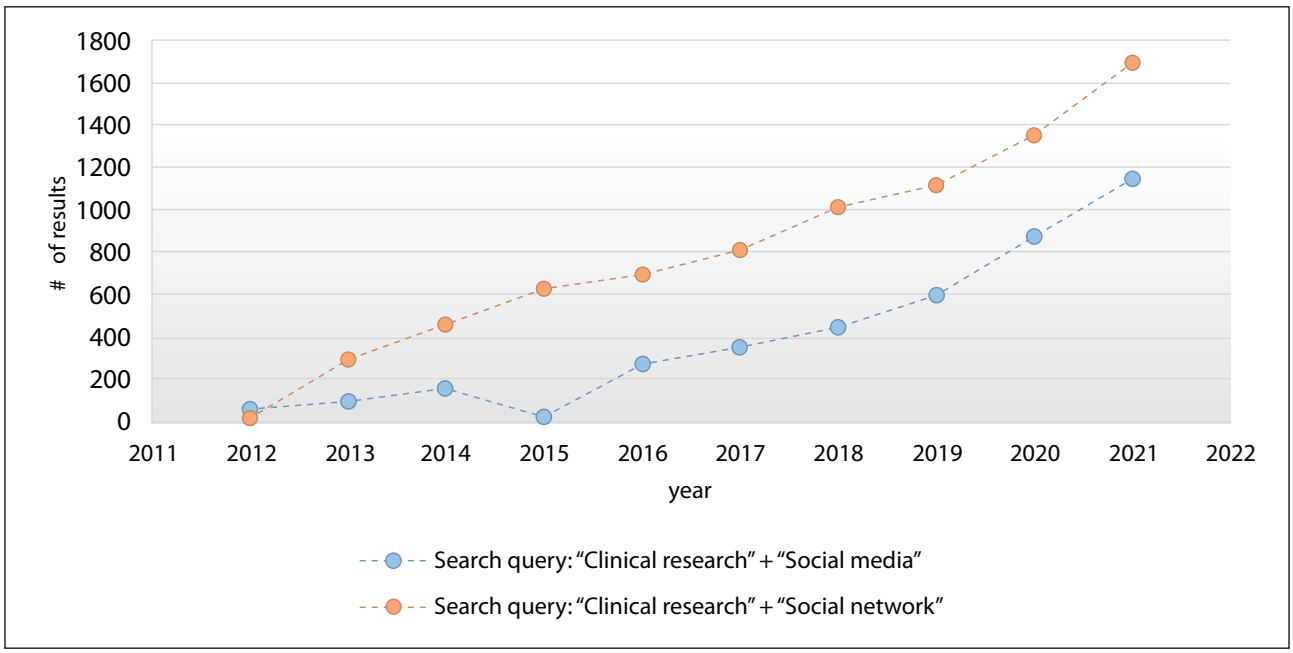

Figure 1. *PubMed search of "clinical research" and "social media" or "social network"*

shows results from the Pubmed search using the following keywords: "clinical research", "social media", "social network".

This type of communication could lead to a greater participation in clinical trials offering patients the best therapeutic options available. Achieving target rates for study recruitment and accrual remains a challenge. Inability to reach eligible patients for recruitment ultimately reduces the statistical power of studies, incurs economic costs, and may jeopardize funding. Evidence suggests that as many as 19% of clinical trials close without meeting at least 85% of target accrual rates, highlighting the need to investigate new methods to implement novel approaches for research recruitment. Study participants report their interest in being involved in the design and implementation of recruitment approaches for clinical research studies. The application of patient-driven platforms and social media could aid in the evolution of clinical research practices for the recruitment of both rare and common diseases, where patient-centric approaches can help create targeted messages designs that participants can pre-test and support (15).

Another positive aspect of this communication is Adverse Events (AE) reporting in clinical trial. The AEs are often reported by patients in specific social groups or networks to share safe information to be discussed: a study has investigated the level of concordance between AE reported through Twitter posts and those received by regulatory agencies. The study concluded that reports on twitter were more accurate, which could be an important consideration to be taken into account in the post marketing safety phase (15).

The international scientific literature is very poor in publications on the use of messaging applications in the health sector. Although its impact on the clinical setting has been poorly investigated, WhatsApp is among the most widely used communication tools, which may also be valuable in favoring the communication and relationship between patients and physicians. Healthcare providers should be trained to use modern web-based communication systems with accurate assessment of indications and contraindications. That said, virtual means should be prevented from replacing real interactions (15). In fact, if the use of social media allows immediate availability and spread of information discussed between different categories, the risk of spreading sensitive data and violating ethical and legal aspects must not be underestimated. The spread, for example, of clinical trial data due to an exchange of information between patients or the share of patient sensitive data could be avoided with guidelines and good practices.

As social media have grown, a gradual, yet extensive-overlap with the medicine field has occurred and continues to evolve. Clinical research has seen extensive social media exposure over the last 5 years, and it is important to understand the implications of this growth (16).

The vast majority of published papers feature investigations conducted among clinicians, while other professional figures are neglected. For this reason, the Italian Group of Data Manager and Clinical Research Coordinator (GIDMcrc), a scientific society that operates in the field of clinical research with a particular focus on oncology, has decided to conduct a project aimed at the various professional figures operating in the field of clinical research.

The project aims to explore, through a questionnaire, the use of social media for working purposes by clinical research professionals and their impressions about the real usefulness of such use.

We also aimed to explore the inclination of patients to contact their physician through social channels and the trend of clinical centers to an institutional use of these means.

MATERIAL AND METHODS

In February 2020 all CRC members of GIDM, at that time accounting for about 200 members, were invited to participate in an anonymous survey, via an email containing the link to complete the questionnaire; all invitations were sent simultaneously, through a mailing list. At the same time, the link was published on the social channels of the GIDM (Facebook and LinkedIn), to reach research professionals other than clinical research coordinators (CRC)/data managers. A copy of the questionnaire is available in **Appendix 1**. The original version of the questionnaire, shared nationally, was Italian.

Participation was voluntary; no reward was offered, nor a fee was requested, for completing the survey. The participation link was active for 40 calendar days and required 15 minutes to complete.

The survey was composed of 13 questions grouped into different investigational sections:

1. demographic and working information (questions 1-3);
2. attitudes toward social media usage (questions 4-6);
3. perceptions on the real usefulness of social media in clinical research (questions 7-8);

4. experience toward the use of social media during their work (questions 9-13) activities.

The attitudes and perceptions of research professionals were assessed by ten-point Likert scales (from "not at all satisfied/strongly disagree" to "extremely satisfied/strongly agree"), by continuous percentage scale or by different answer options.
Before producing the final version, an initial draft (version 0) was delivered as a preliminary test to 15 clinical research professionals: 5 CRC, 5 study nurses (SN) and 5 medical doctors (MD). The comments and corrections collected were implemented giving way to a new version (version 1) delivered to 6 more clinical research professionals with homogeneous distribution among the different types of institutes. This new test represented the final version of the questionnaire used in this project.
The survey did not include fields for identification of participants, including information on specific age (only age range) and sex, or their specific institution. Collection of surveys, calculation of results, and data entry on a password-protected electronic database were performed by a third operator who had no access to information regarding GIDM members' emails addresses or identities.
Characteristics of stakeholders who participated were analyzed using descriptive statistics and results were reported as the absolute number of respondents for each answer option on the total number of people responding to that specific question. When more than one option was allowed, the sum of percentages for each given answer was >100%. Data were analyzed in January 2021 and as per national law, the project was not evaluated by an ethics committee.

RESULTS

On 15 April 2020, 100 research professionals completed the survey (**table I**), mostly clinical research coordinators (n = 52, 52%), physicians (n = 23, 23%) or nurses (n = 20, 20%) (**figure 2**).
The most represented age group is 30-49 years (n = 79, 79%), with only one respondent over 65 years of age (**figure 3**). The respondents' average work experience is 11 years, with a considerable share of professionals with experience over 5 years (n = 66, 66%).
Only a small proportion of stakeholders (n = 13, 13%) said they do not use social media to search and/or share information while the majority use them often (n = 33, 33%) or sometimes (n = 34, 34%).

Table I. *Characteristics of respondents.*

CHARACTERISTICS	N (%)
Age (years)	
18-29	8 (8%)
30-49	79 (79%)
50-65	12 (12%)
>65	1 (1%)
Profession	
Clinical Research Coordinator/Data Manager	52 (52%)
Physician	23 (23%)
Nurse	20 (20%)
Other	5 (5%)
Years of profession	
≤5	34 (34%)
6-24	56 (56%)
≥25	10 (10%)

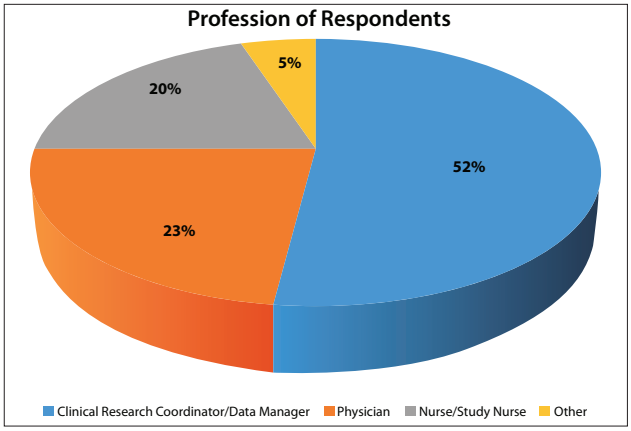

Figure 2. *Question 2 - Profession of respondents (total # respondents: 100).*

Among the social networks proposed by the authors, the one best known / used for business purposes by the total number of respondents is Facebook (n = 65, 74.7%), followed by LinkedIn (n = 60, 69.0%).
The "supremacy" of these two social networks is seen in all age groups while Research Gate seems to be particularly appreciated in the 30-49 years range (n = 22, 32.8% *vs.* 12.5 in 18-29 years and 25.0% in the 50-65 years' groups). On the contrary, Instagram is not very appealing to the 30-49 age range (19.4%) while it seems to be more appreciated by respondents aged 18-29 (37.5%) and even more so by those aged 50-65 (41.7) (**table II**).
Stratifying by professional figure, Facebook remains the preferred social network for CRC and SN (n = 42, 80.7% and n = 15, 75% respectively) while clinicians seem to prefer ResearchGate, albeit slightly (n = 12, 52.2%) (**table III**).
Of the 6 social media suggested by the authors, a minority of respondents know/use only one (n = 23,

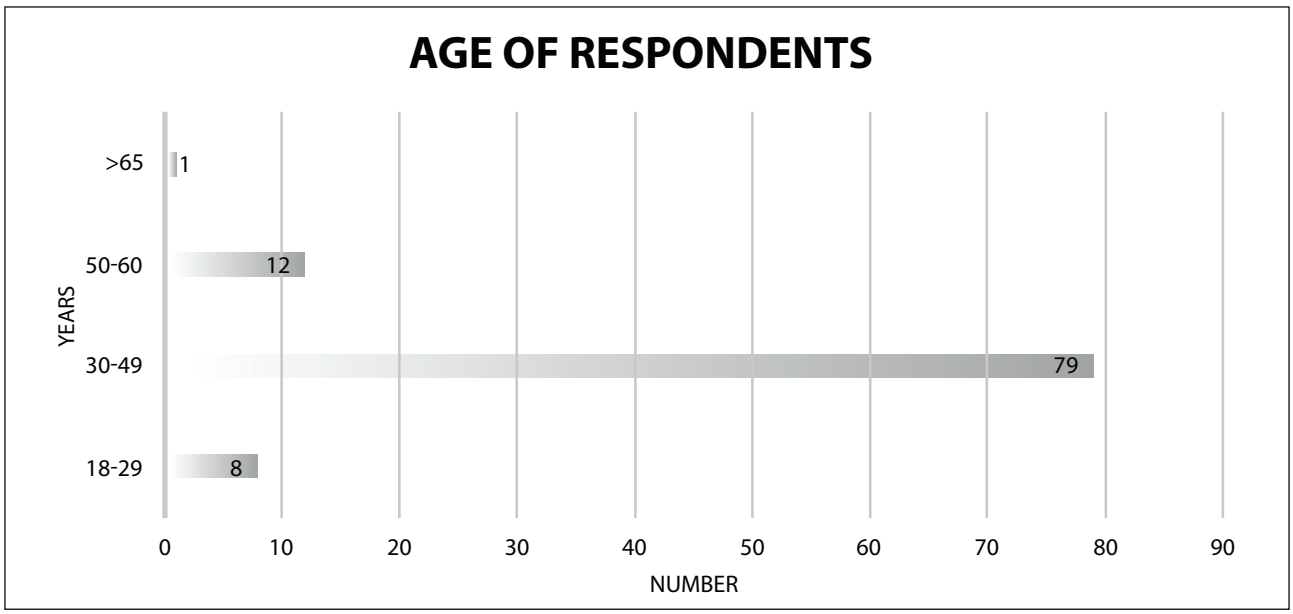

Figure 3. *Question 1 - Age of respondents (total # respondents: 100).*

Table II. *Social media used for age groups.*

KNOWN/UTILIZED SOCIAL MEDIA	ALL AGE GROUPS (SAMPLE: 87) N (%)	18- 29 YEARS (SAMPLE: 8) N (%)	30-49 YEARS (SAMPLE: 67) N (%)	56-65 YEARS (SAMPLE: 12) N (%)
Facebook	65 (74.7%)	4 (50.0%)	55 (82.1%)	5 (41.7%)
LinkedIn	60 (69.0%)	4 (50.0%)	50 (74.6%)	6 (50.0%)
Instagram	20 (23.0%)	3 (37.5%)	13 (19.4%)	5 (41.7%)
Research Gate	26 (29.9%)	1 (12.5%)	22 (32.8%)	3 (25.0%)
Twitter	18 (20.7%)	2 (25.0%)	14 (20.9%)	4 (33.0%)
YouTube	15 (17.2%)	2 (25.0%)	9 (13.4%)	4 (33.0%)
Others	2 (2.3%)	0 (0%)	2 (3.0%)	0 (0%)

23%) or two (n = 27, 27%). Two respondents communicated that they also use social networks other than those proposed (1 Google and 1 WhatsApp).

The interviewed stakeholders declared that they use social media mainly to find information (n = 37, 37%) or to find and share it in equal measure (n = 36, 36%) (**figure 4**). The same trend is confirmed if the data is stratified by professional figure, with a very slight preference in the search for information rather than sharing for CRCs and MD.

The evaluation of the respondents with respect to the real usefulness of social networks for professional purposes is average (average score 5.93 on a 1-10 scale), without major differences between the different age groups (5.90 in 18-29 years' group; 5.93 in 30-49; 5.66 in 50-55) and professional categories (5.93 CRC, 5.51 SN, 5.97 MD).

The evaluation of the usefulness of social media reported by cancer patients is also average (average score: 6.00), with a pessimism that characterizes above all 50-65 age-range group of respondents (5.18 *vs.* 5.86 in 18-29 years' and 6 in 30-49 years' group). No significant differences emerged between the different professional groups, with a median score of 6.0 for CRC, 5.68 for SN and 5.9 for MD.

Almost half of the respondents (n = 43, 43%) have been contacted by patients through social networks. The preferred method by patients is Facebook (n = 26, 60.5%), followed almost equally by WhatsApp (n = 16, 37.8%) and e-mail (n = 18, 41.9%). In addition, three professionals said they had been contacted by their patients through LinkedIn.

As regards the position of the Clinical Centers with respect to the official use of social net-

Table III. *Social media used for professional figure.*

KNOWN/UTILIZED SOCIAL MEDIA	CLINICAL RESEARCH COORDINATORS (SAMPLE: 52) N (%)	MEDICAL DOCTORS (SAMPLE: 23) N (%)	STUDY NURSES (SAMPLE: 20) N (%)	OTHERS (SAMPLE: 5) N (%)
Facebook	42 (80.8%)	11 (47.8%)	15 (75.0%)	3 (60.0%)
LinkedIn	39 (75.0%)	11 (47.8%)	6 (30.0%)	5 (100%)
Instagram	5 (9.6%)	5 (21.7%)	9 (45.0%)	2 (40.0%)
Research Gate	13 (25.0%)	12 (52.2%)	4 (20.0%)	2 (40.0%)
Twitter	4 (7.7%)	11 (47.8%)	4 (20.0%)	1 (20.0%)
YouTube	5 (9.7%)	3 (13.0%)	6 (30.0%)	0 (0%)
Others	2 (3.9%)	0 (0%)	0 (0%)	0 (0%)

works, the answers show that less than half (n = 48, 48%) use e-mails or other messaging systems (*e.g.*: WhatsApp) for patient communication and/ or appointment management. The management of these services is very heterogeneous in the different Centers, but often involves the direct involvement of clinicians (n = 16, 33.3%) or support staff (nurses: n = 11, 22.9%; Clinical Research Coordinators/Data Managers: n = 9, 18.7%). There was also a case of implementation of a specific communication office responsible for this service.

DISCUSSION AND CONCLUSIONS

In this article, we attempted to examine the use of social media among professionals involved in clinical research. Social media is flawed, but at its best it offers a way to navigate an ever-shifting cultural climate (16) and this was especially clear in the era of the COVID-19 pandemic (6).

Through this survey, we were able to examine the current attitudes of clinical research professionals – not only clinicians – toward social media in many different aspects. Although our target audience were mostly clinical research coordinators (half of participants), the survey also included physicians,

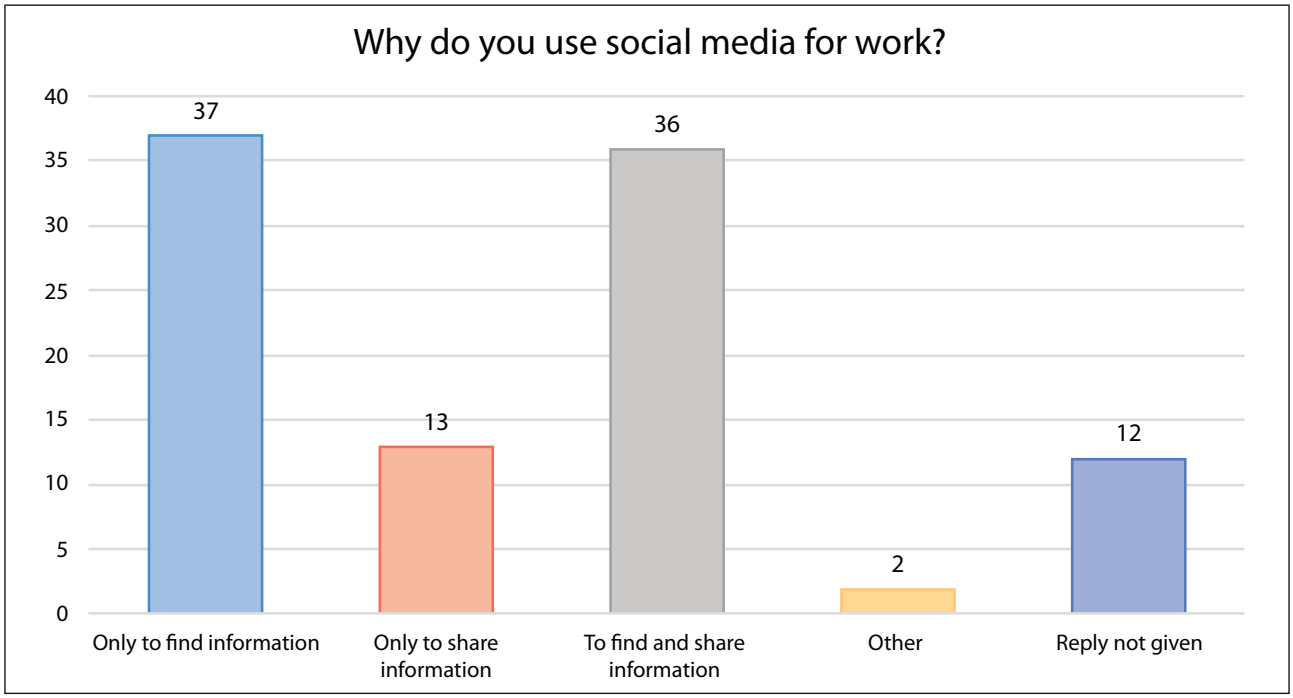

Figure 4. *Question 6 - Use of social media for work purposes (total # respondents: 100).*

nurses and other professionals involved in clinical research, giving a good insight into the multidisciplinary groups operating in the field of clinical research. Almost 80% of these respondents have been practicing for at least 10 years, with a considerable share of professionals with experience over 25 years, between the age of 30 and 49 years.

Our data confirm the trend already highlighted by literature for clinicians: social networks are valued and their use for professional purposes is growing and is destined to expand. The survey highlights that Facebook (75%) and LinkedIn (69%) were the preferred platforms and most professionals use social networks only to search for information (37%) or both to find and share information (36%). Interestingly, we were not expecting the low percentage of use of Twitter; in our experience, in fact, Twitter is a very valued media used by physicians as a means for sharing abstracts/posters presented during the most esteemed international congresses. This data should, however, be interpreted taking into consideration the low percentage of clinicians among the respondents, compared to other professional figures.

In general, clinicians and researchers can use social media professionally for two purposes. Firstly, social media serves as an information aggregator, helping users stay up to date on relevant advances in the medical field. Secondly, social media serves as an engagement tool, helping users to connect with others who have similar interests, to foster collaboration, and to gain support for personal and professional growth (13).

The widespread use, that in part seems to clash with the perception of real usefulness, reaches average scores (no more than 6 points out of 10) even by stratifying by age and professional categories. Very similar is the impression as regards the usefulness of social media reported by patients: also, in this case the answers of the professionals are very cautious.

This probably reflects the awareness that, net of the potential, social media still have numerous critical issues: ethical concerns (17), low communication barriers, limited privacy, and security issues (18), professionals' unawareness of workplace policy on the use of social media (19) and a real risk of misuse by physician (20).

Even more worrying are those issues regarding the use of social media by patients, who are unfortunately still often victims of rumours and misinformation (21, 22). Patients are also exposed through social media to the destructive phenomenon of fake news, which was especially evident in the last two years during the Covid-19 pandemic.

Despite this, we are inclined to recommend that clinicians and researchers follow a diverse set of reputable health organizations, established scientists, or journal clubs to stay current with reliable research and participate in scheduled live-chats, where users discuss health-related topics (23).

Social media can help break down traditional barriers that prevent interaction between healthcare professionals as well as between providers, scientists, patients, and caregivers (13).

Half of the interviewees were contacted by patients looking for information or for managing their appointments. However, all this is done without specific training. If patients, healthcare professionals and researchers were informed and instructed on the correct use of social network, there could be benefits for research, such as faster recruitment timelines in clinical trials, involvement of patients in the study design, and sharing of trial results.

Given the popularity and almost universal appeal of social media, we encourage physicians and institutions to learn and engage more in this ongoing evolution. Protection of patients as well as physicians is critical. Further research, collaboration, and funding are needed to improve the evidence base to determine how we can effectively leverage social media to engage patients, providers, and communities to improve health behavior and outcomes. The protection of the institution's and physicians' reputations as well as patient privacy needs to be carefully safeguarded. Physician protection would extend to the separation of personal and professional use of social media. In addition, the importance of transparency cannot be overestimated. Any involvement by physicians in social media, whether it is personal or professional, if not entirely transparent, can lead to repercussions on institutions or professional reputations. Even though a number of uses for social media have come to the forefront, it is undeniable that new and unforeseen uses, benefits, and potential concerns will arise in the future (24).

It is evident that a steadfast commitment (and investment by the institutions) is necessary for the technological training of citizens and workers, which in some countries, such as Italy, is very weak. Probably in the future, as proposed by some authors, specific guidelines will also be needed (25).

Certainly, our research has several limitations, such as the small sample and the national diffusion. Another important limitation is the fact that it is not possible to estimate total number of professionals reached with the invitation to reply to the survey. In addition, there might be a discordance between the respondents and the actual sample, meaning that the professionals responding to the survey might correspond mostly to those with a greater interest in social media, as opposed to others less familiar with these tools. Therse are limitations commonly seen in literature for this type of research/investigation modality.

That said, we think it represents an important point of view in the world of clinical research and leads us to believe that social media engagement may be a valuable tool to advance healthcare professionals' own growth and foster patient engagement and education.

COMPLIANCE WITH ETHICAL STANDARDS

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The Authors have declared no conflict of interests.

Availability of data and materials

The data underlying this article are available in the article.

Authors' contributions

ST, CC: conceptualization; ST, CC: methodology; VF, FM: validation; CC: formal analysis; ST, CC, VF; ST, CC, RC: writing/review and editing.

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Human studies and subjects

N/A.

Animal studies

N/A.

Publications ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

N/A.

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APPENDIX 1

When research becomes "social": not only fake news

Questionnaire

1) What is your age range?

- 18-29
- 30-49
- 50-65
- >65

2) What is your profession?

- Doctor
- Nurse
- Clinical Research Coordinator/Data Manager
- Other:.....

3) State your professional experience in years:

- .....

4) How often do you use social media to search/share information concerning your professional activity?

- Never (skip to question 7)
- Rarely
- Sometimes
- Often

5) Which of these social media are you familiar with and use to this end?

- Facebook
- Linkedin
- Instagram
- Researchgate
- Twitter
- Youtube
- Other:.....

6) When using social media for professional purposes, your primary aim is to:

- Gain information
- Share information
- Gain and share information to the same extent
- Other:.....

7) From 0 (not at all) to 10 (very), how useful do you rate social media to be in your professional activity?

- .....

8) From 0 (not at all) to 10 (very), how useful do you rate social media to be for patients with cancer?

- .....

9) Have you ever been contacted by a patient through social media?

- Yes
- No

10) If yes, via:

- E-mail
- Whatsapp
- Facebook
- Other:.....

11) Does your institution use e-mail or other messaging service (whatsapp or other) to communicate with patients and/or schedule visits?

- Yes
- No

12) If yes, who carries out this task?

- Doctor
- Nurse
- Data Manager/Clinical Research Coordinator
- Other:.....

13) At your institution, have specific courses/training sessions on social media been held for healthcare and research personnel?

- Yes
- No