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Review Article

Therapeutic targeting of the protein tyrosine kinase-7 in cancer: an overview

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ABSTRACT

The protein tyrosine kinase-7 (PTK7) is an evolutionarily conserved transmembrane receptor that has emerged as a potential therapeutic target for human tumors. PTK7 is a pseudokinase that is involved in the modulation of the Wnt signaling pathway through interactions with other receptors. These interactions result in targeted gene activation that regulates cell polarity, migration, and proliferation during embryogenesis. Aside of this role during development, PTK7 has been shown as overexpressed in numerous cancers including colon carcinoma, leukemia, neuroblastoma, hepatoma, and ovarian cancer. The activity of PTK7 and the direct correlation with poor prognosis have fostered preclinical investigations and phase I clinical trials, aiming at inhibiting PTK7 and inducing antitumoral effects. In this review, we provide an exhaustive overview of the diverse approaches that use PTK7 as a new molecular target for cancer therapy in different tumor types. We discuss current therapies and future strategies including chimeric antigen receptor-T cells, antibody-drug conjugates, aptamers, based on up-to-date literature and ongoing research progress.

Key words: PTK7; cancer therapy; targeted therapy; CAR-T cell; antibody-drug conjugate; aptamer.

Implications for practice

Protein tyrosine kinase-7 (PTK7) has been proposed as a therapeutic target for different blood malignancies and solid tumors. Increasing amount of data sheds light on the role PTK7 could play in cancer, as well as its great potential as a tumor specific marker. PTK7 targeting has been proved efficient in various preclinical studies and has also entered clinical validation in different phase I and II trials. In our paper, we gathered recent data in a very exhaustive manner, to discuss the wide panel of therapeutic approaches that have so far been considered for targeting PTK7.

Introduction: protein tyrosine kinase 7, expression and function in human physiology and malignancy

The protein tyrosine kinase 7 (PTK7) is a member of the receptor protein tyrosine kinase (RTK) family, playing a crucial role in several cellular signaling cascades. Initially identified as upregulated in colon cancer, PTK7 is also known as colon carcinoma kinase 4.[1] Seven different transcript variants, including 5 protein-coding, arise from alternative splicing of the human PTK7 gene.

RTKs are cell surface receptors that mediate various cellular processes by transmitting extracellular signals into the cell, ultimately regulating cell growth, differentiation, and survival.[2] RTK dysregulation is a common event in cancer. Mutations, gene amplification, or aberrant activation of these receptors can lead to uncontrolled cell proliferation, evasion of apoptosis, angiogenesis, invasion, and metastasis, all hallmarks of cancer progression. Consequently, targeting RTKs has long been considered as a promising therapeutic strategy in various cancers, for example, in lung or breast cancer, using small molecules or antibodies, to disrupt oncogenic signaling and impede tumor growth and metastasis.[3]

Typically, RTKs exhibit a tripartite structure comprising an extracellular ligand-binding domain, including 7 immunoglobulin domains, a transmembrane domain, and an intracellular tyrosine kinase domain. PTK7, however, stands out from classical RTKs, due to its notable deficiency in conventional tyrosine kinase catalytic activity, and is therefore characterized as a defective kinase or a pseudokinase.[1,4] However, PTK7 emerges as a central player in orchestrating developmental processes and maintaining tissue homeostasis through the modulation of the Wnt signaling. In the canonical Wnt pathway, PTK7 interacts with key receptors, including Frizzled and LRP5/6, facilitating the transmission of Wnt signals into the cell. These interactions triggers a signaling cascade that ultimately leads to the stabilization and nuclear translocation of β-catenin, which then associates with TCF/LEF transcription factors to activate target genes involved in processes like

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cell proliferation, differentiation, and embryonic patterning.[5] Concurrently, in the non-canonical Wnt pathway, PTK7 interactions with other pseudokinase receptors such as Ror1/2 and Ryk contribute to the regulation of planar cell polarity, cell migration, and embryogenesis.[6,7] Dysregulation in the PTK7-Wnt signaling axis has been linked to various developmental defects.[8]

PTK7 expression in normal adult tissues has been shown as generally low,[1,9,10] however the aberrant upregulation of PTK7 has been involved in several cancers, pointing to its significance as a potential prognostic marker and therapeutic target in oncology (Figure 1). An increased expression of PTK7 has indeed been observed in diverse malignancies, including but not limited to colon cancer, breast cancer, ovarian cancer, and gastric cancer, as also assessed by immunohistochemistry in different studies. This high expression of PTK7 in diverse cancer tissues has so far not been associated to copy-number alterations, for example, amplification or duplication events in the *PTK7* gene, as none of these were described. However, both experimental evidence and clinical data show that PTK7 upregulation is often associated with aggressive tumor behavior, enhanced metastatic potential, and resistance to conventional therapies, thereby correlating with a poor prognosis for patients.[8] Interestingly, one germline mutation variant in *PTK7* (resulting in PTK7^{V354M}) has been identified in a small cohort of patients with familial colon cancer, and suggested to confer even higher aggressiveness to cancer cells.[11]

Understanding the implications of PTK7 upregulation and putative genomic alterations in specific cancer types is crucial for refining diagnostic and prognostic strategies. Furthermore, elucidating the molecular mechanisms underlying PTK7 dysregulation in cancer may unveil novel avenues for targeted interventions and personalized treatment approaches aimed at improving the outcome of patients combatting solid tumors. In this review, we depict the landscape of PTK7-targeted therapies in cancer, encompassing a diverse array of innovative approaches including chimeric antigen receptor (CAR) T cells, antibody-drug conjugates (ADCs), aptamers, among most described approaches (Figure 2). Most studies described below are summarized in Table 1.

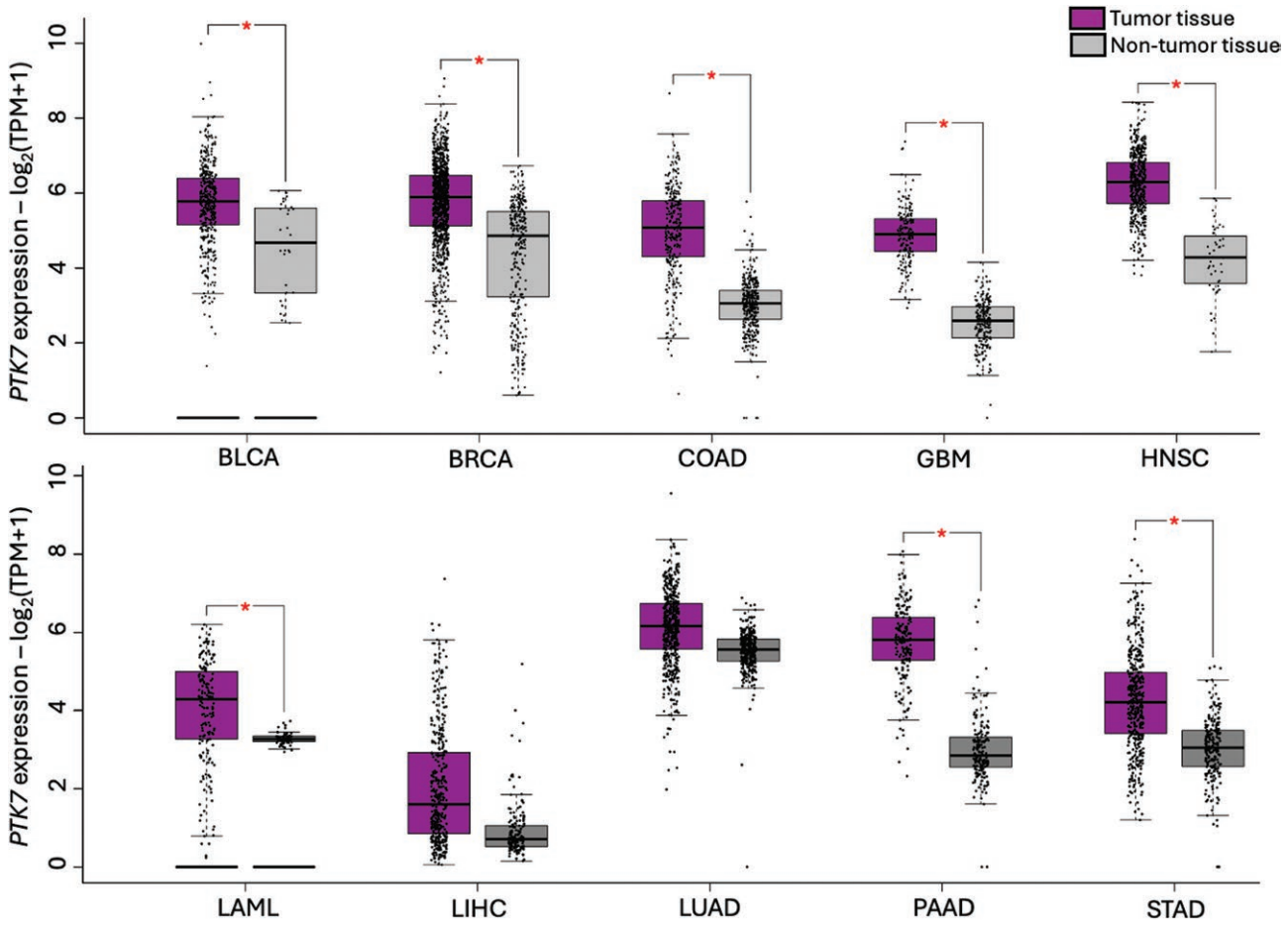

Figure 1. Expression of PTK7 in different tumor (T) types, compared to the corresponding non-tumoral (N) tissues. These graphs were obtained from the GEPIA2 online tool, providing gene expression analysis based on the RNA sequencing data from the Cancer Genome Atlas datasets and GTex normal tissue database. Gene expression in tumor samples (T) and non-tumoral samples (N, gray) is displayed as log2 (TPM + 1). Each dot represents one patient sample. Sample size in each group (num) is indicated below the graphs. Abbreviations:BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; GBM, glioblastoma; HNSC, head-and-neck squamous cell carcinoma; LAML, acute myeloid leukemia; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; PAAD, pancreatic adenocarcinoma; STAD, stomach adenocarcinoma; TPM, transcripts per million.

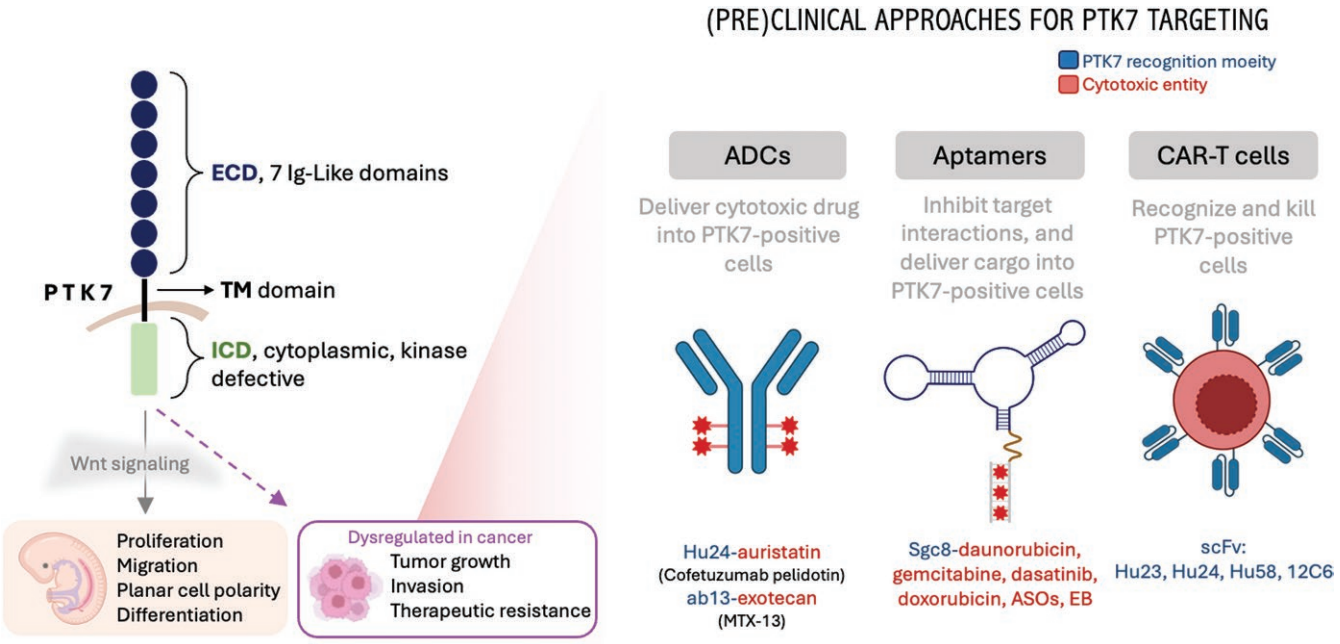

Figure 2. Architecture and roles of PTK7 in physiology and in cancer, and overview on the 3 main targeting approaches. The protein tyrosine kinase 7 (PTK7) is composed of 7 Ig-like domains forming the extracellular domain (ECD), one transmembrane (TM) domain followed by an intracellular domain (ICD) without effective kinase activity. PTK7is involved in proliferation, migration, planar cell polarity, and differentiation by modulating the Wnt signaling pathway. In the context of cancer, PTK7 is involved in tumor growth, invasion, and therapeutic resistance. Experimental and clinical strategies for targeting cancer cells via PTK7 include antibody-drug conjugates (ADCs), aptamers and CAR-T cells. Each approach generally combines (1) a PTK7 recognition moeity that can be an immunoglobulin (Ig), a single domain antibody (scFv), or a DNA aptamer; with a (2) cytotoxic entity that can be a chemotherapeutic payload, an antisense oligonucleotide (ASO), or the cytotoxic lymphocyte itself. Figure designed using BioRender software.

Therapeutic approaches based on PTK7 targeting

Monoclonal ADCs

To date, over a hundred monoclonal antibodies (mAbs) have been approved by the Food and Drug Administration (FDA),[30] a third of them used for cancer treatment in the context of immunotherapy or for precise targeting of tumor surface antigens. The groundbreaking development of antibody-drug conjugates (ADCs) connected the high specificity of mAbs to the efficacy of cytotoxic drugs. Nowadays, 14 ADCs are FDA-approved and many others are currently in clinical trials.[31] ADCs associate a tumor-targeting monoclonal antibody linked to a cytotoxic payload thanks to a chemical linker, allowing for precise and specific cancer cell targeting and potent effectiveness concurrently, with minimized side effects.[32]

The cofetuzumab pelidotin is a humanized anti-PTK7 ADC (hu6M024 or Hu24, IgG1) designed to deliver an auristatin microtubule inhibitor cargo (auristatin-0101) into target cells. This ADC demonstrated cytotoxic action, microtubule disruption, mitotic arrest of ovarian cancer cells (OVCA), non-small cell lung cancer cells (NSCLC), small cell lung cancer cells (SCLC). and triple-negative breast cancer cells (TNBC) as well as reduced tumor size in all models.[10] Target-dependent toxicity was tested in cynomolgus monkeys and indicated a safe profile of cofetuzumab pelidotin, which warranted further clinical testing. In a phase I study, this anti-PTK7 ADC was injected in patients with advanced solid tumors resistant to available standard therapy.[12] The maximal tolerated dose of cofetuzumab pelidotin was defined between 2.8 and 3.7 mg/kg every 3 weeks, and most treatment-related adverse events were acceptable with a dose-limiting toxicity interval of 20%-30%, and half-life of 3 days. Although the trial included small patient cohorts, objective tumor responses were observed ranging from 26% to 27% in OVCA, 16%-33% in NSCLC, and 21% in patients with TNBC.

A couple of studies associate this ADC with other cancer treatments. Radovich et al, used cofetuzumab pelidotin in combination with gedatolisib, a PI3K pathway inhibitor, in a phase I study in TNBC patients. Indeed, gedatolisib induces a compensatory Wnt signaling which leads to PTK7 upregulation and metastasis, hence is not sufficiently efficient as a single treatment. However, the synergic treatment demonstrates a clinical benefit.[14] In OVCA cells, cofetuzumab association with paclitaxel (inhibitor of microtubule degradation) or prexasertib (checkpoint kinase inhibitor) showed increased effect on cell viability, suggesting that targeting PTK7 may also improve the response to these drugs.[13]

Arguing on the systemic toxicity and limited efficacy of auristatin-0101 payload, a novel anti-PTK7 ADC, MTX-13, has recently been developed based on a new PTK7-targeting antibody (Ab13) conjugated to 8 molecules of topoisomerase I inhibitor exotecan.[15] This new anti-PTK7 ADC with suitable safety and pharmacokinetics profile demonstrates a correct internalization into PTK7-positive cancer cells and shows good cytotoxicity, inducing DNA damage and apoptosis. Injection of MTX-13 (5 mg/kg or 10 mg/kg) in different tumor models (eg, OVCA, NSCLC, and TNBC) in mice or cynomolgus monkeys demonstrated a better antitumor activity compared to cofetuzumab pelidotin. Efficient inhibition of tumor growth and metastasis in colon cancer was also obtained with MTX-13, even at an advanced stage of the disease. In a SCLC patient-derived xenograft (PDX) model, one dose of MTX-13 caused tumor regression for approximately

Table 1. Overview of the PTK7 therapeutic targeting approaches in cancer, in preclinical, and clinical studies.

Cancer type	Models	Approach for specific targeting	Therapeutic modalities	Impact of PTK7 targeting	Ref
Anti-PTK7 antibody-drug conjugates (ADC)					
- Ovarian cancer (OVCA) - Non–small cell lung cancer (NSCLC) - Small cell lung cancer (SCLC) - Triple-negative breast cancer (TNBC)	*In vitro*: Human cell lines - OVCA: OVCAR3 - NSCLC: H661 - SCLC: H446 *In vivo*: - S.c. xenograft mouse model - Monkey model	**Cofetuzumab pelidotin** (PF-06647020): - *Antibody*: Humanized, anti-PTK7 antibody (hu6M024 or Hu24, US patent US20150315293A1) - *Conjugate*: auristatin (Aur0101)	*In vitro*: Application in the culture medium *In vivo:* - Mouse: Intraperitoneal injection twice a week for 4 cycles with PTK7-targeted ADC (1 to 3 mg/kg) or control (nonbinding) ADC or with standard-of-care chemotherapy. - Monkey: Repeated doses (once every 3 weeks for 3 cycles with doses up to 5 mg/kg).	*In vitro*: - ↑ cytotoxicity - ↑ microtubule disruption and mitotic arrest *In vivo* (mouse): - Tumor cell cycle arrest - ↓ tumor growth - No effect of unconjugated Abs - Depletion of tumor-initiating cells *In vivo* (monkey): - No indication of target-dependent toxicity.	[10]
- Platinum-resistant ovarian cancer (OVCA) - Non-small cell lung cancer (NSCLC) - Triple-negative breast cancer (TNBC)	*Phase I study:* Patients with advanced solid tumors resistant to or with no available standard therapy	**Cofetuzumab pelidotin** (PF-06647020): - *Antibody*: Humanized, anti-PTK7 antibody (hu6M024 or Hu24, US patent US20150315293A1) - *Conjugate*: auristatin (Aur0101)	(*Prior anticancer therapy*). i.v. injection: - every 3 weeks at doses ranging from 0,2 to 3,7 mg/kg - every 2 weeks at 2,1, 2,8, and 3,2 mg/kg, in sequential dose-escalation cohorts. *(until disease progression, unacceptable toxicity, or withdrawal of consent)*	- Mild to moderate treatment-related adverse events - Overall results similar in the 2 groups - Objective tumor responses were observed, ranging from 26% to 27% (OCVA), 16% to 33% (NSCLC) and 21% (TNBC).	[12]
Ovarian cancer (OVCA)	*In vitro:* - PDCs from both type I and II	**Cofetuzumab pelidotin** (PF-06647020): - *Antibody*: Humanized, anti-PTK7 antibody (hu6M024 or Hu24, US patent US20150315293A1) - *Conjugate*: auristatin (Aur0101)	*In vitro*: Application in the culture medium	*In vitro*: - ↓ Tumor cell proliferation. - ↑ Tumor suppressor molecular effectors - ↓ Pro-tumoral molecular effectors - Interference with migration and epithelial-mesenchymal transition processes	[13]
- Triple-negative breast cancer (TNBC) - Estrogen Receptor-low breast cancer (BrC)	*Phase I study:* Patients with metastatic TNBC or ER-low BrC	**Cofetuzumab pelidotin** (PF-06647020): - *Antibody*: Humanized, anti-PTK7 antibody (hu6M024 or Hu24, US patent US20150315293A1) - *Conjugate*: auristatin (Aur0101) + Gedatolisib: Inhibitor of Pl3K/mTOR	(*Prior first-line treatment*) i.v. injection: - Gedatolisib (110 to 180mg, at D1, D8, D15 and every 21 days) - Cofetuzumab pelidotin (1.4 to 2.8 mg/kg at D1 and every 21 days).	- Mild to moderate adverse events - Overall response rate: 16.7% - Clinical benefit at 18 weeks: 27.8% - Progression-free survival: 2 months - Genomic alterations in PI3K and PTK7 pathways	[14]

Table 1. Continued

Cancer type	Models	Approach for specific targeting	Therapeutic modalities	Impact of PTK7 targeting	Ref
- Ovarian cancer (OVCA) - Triple-negative breast cancer (TNBC) - Hypopharyn-geal carcinoma (HC) - Stomach cancer (SC) - Non-Small Cell Lung Cancer (NSCLC) - Small cell Lung Cancer (SCLC) - Esophageal carcinoma (EC) - Pancreatic cancer (PC) - Colon cancer (CC)	*In vitro*: Human cell lines/ cultures: - OVCA: OVCAR-3, SK-OV-3 - TNBC: MDA-MB-468, PDCs - HC: FaDu - SC: SNU-16 - NSCLC: PC-9, NCI-H2170, NCI-H226, NCI-H520, NCI-H1975 - SCLC: PDCs - EC: KYSE-150 - PC: BxPC-3 - CC: HCT116 *In vivo*: - S.c. xenograft mouse model - Monkey model	**MTX-13**: - *Antibody*: Humanized, anti-PTK7 antibody (ab13) - *Conjugate*: exatecan	*In vitro*: Application in the culture medium *In vivo* (mouse/monkey): Transplantation of different tumor lines with oral administration of MTX-13.	*In vitro:* - Internalization of MTX-13 - Efficient cytotoxicity *In vivo (mouse)*: - ↓ Tumor growth (better efficacy of MTX-13 vs PF-06647020 - Wider therapeutic index *In vivo (monkey):* Well tolerated. Toxicity was observed in bone marrow, thymus, liver, gallbladder and stomach.	[15]
Anti-PTK7 CAR-T cells					
Neuroblastoma (NB)	*In vitro*: Human cell lines/ cultures: - NB: SMS-SAN, KELLY, LA-N-5, SK-N-AS, SK-N-SH, IMR5, NGP, NLF, SK-N-BE(2)-C - Osteosarcoma: 143B, MG-63 - Rhabdomysarcoma: Rh41 *In vivo*: - S.c. xenograft mouse model	- *CAR*: Humanized, anti-PTK7 scFv (clone 12C6 from US Patent 9102738B2) - *Cells*: Jurkat	*In vitro*: Co-cultures PTK7 CAR-T cells + Human NB cells, with treatment (topotécan + cyclophosphamide) *In vivo*: Human NB cells graft, with treatment (topotécan + cyclophosphamide)	*In vitro*: - ↓ T cell activation - ↑ Cytotoxicity *In vivo:* - ↑ Survival - ↓ Tumor size - No undesired effects - PTK7 expression maintained at recurrence	[9]
- Non–small cell lung cancer (NSCLC) - Small cell lung cancer (SCLC)	*In vitro*: Human cell lines/ cultures: - NSCLC: H520, H1975, H1299 - SCLC: H446, H69 - PC: BxPC3 - BrC: MDA-DB-468 - OVCA: OVCAR3 *In vivo*: - S.c. xenograft mouse model	- *CAR*: Humanized, anti-PTK7 scFv 1-2-3 (VH/VL sequences from Hu23, Hu24, and Hu58; US patent US20150315293A1) - *Cells*: Primary T cells	*In vitro:* Co-cultures PTK7 CAR-T cells + different human cancer cell lines *In vivo:* Human NSCLC, SCLC cells graft. 7 & 14 days post-graft: Two injections of control T cells or PTK7-CAR2	*In vitro*: - ↑ Production of IFN-γ and IL-2 by CAR-T cells - ↑ CAR T-cell proliferation - ↑ Tumor cell cytotoxicity (incl. $PTK7^{low}$ cells) *In vivo:* - ↑ Survival - ↓ Tumor size - No undesired effects - ↑ Accumulation of T lymphocytes in tumor	[16]
Ovarian cancer (OVCA)	*In vitro*: Human cell lines/ cultures: - OVCA: OVCAR3, SKOV3 *In vivo:* - S.c. xenograft mouse model	- *CAR*: Humanized, anti-PTK7 scFv 1-2-3 (VH/VL sequences from Hu23, Hu24, and Hu58; US patent US20150315293A1) - *Signal transduction*: TREM1/ DAP12 - *Cells*: Primary T cells	*In vitro*: Co-cultures CAR-T cells and Human OVCA cell lines *In vivo*: SKOV3 cells graft and injection of a single dose of PTK7 CAR-T cells or non-transduced T cells	*In vitro*: - ↑ Tumor cell cytotoxicity - ↑ CAR T-cell proliferation *In vivo:* - Complete tumor eradication - No tumor recurrence - No adverse effects	[17]

Table 1. Continued

Cancer type	Models	Approach for specific targeting	Therapeutic modalities	Impact of PTK7 targeting	Ref
Anti-PTK7 aptamers					
Acute lymphoblastic leukaemia (ALL)	*In vitro*: Human cell lines/ cultures: - ALL: Molt-4 - Myeloma: U266 (PTK7 negative control)	**Dau:Sgc8** - Sgc8 aptamer - *Cargo*: daunorubicin	*In vitro*: Application in the culture medium	*In vitro*: - Efficient and specific internalization in PTK7$^+$ cells - ↓ Cytotoxicity in PTK7- cells - = cytotoxicity in PTK7$^+$ cells compared to Dau alone	[18]
Bladder cancer (BC)	*In vitro:* - Human BC cell line (BIU87, 5637, T24, EJ, RT4, J82, UM-UC-3, TCCSUP) - Normal bladder uroepithelial cell line *In vivo*: - S.c. xenograft mouse model - Mouse model of lung metastasis: i.v. injection of tumors cells - Rat model of in situ bladder cancer: infusion of their bladders with tumors cells	**PTK7-GEM** - Sgc8 aptamer - *Cargo*: gemcitabine	*In vitro*: Application in the culture medium *In vivo*: Injections of PTK7-GEM, 1 every 2 days	*In vitro*: - ↑ Recognition of BC cells (vs normal cells) *In vivo*: - ↓ Tumor proliferation (vs GEM alone) - ↑ Cytotoxicity (vs GEM alone) - ↓ Metastasis (vs GEM-treated groups) - Good biosafety	[19]
Acute lymphoblastic leukaemia (ALL)	*In vitro:* Mouse cell line - B lymphoma: A20 Human cell lines - ALL: CCRF-CEM - GBM: U87 (PTK7 negative control)	**Sgc8-c-carb-da** - Sgc8 aptamer - *Cargo*: dasatinib	*In vitro*: Application in the culture medium	*In vitro*: In human and mouse models: - ↑ Cytotoxicity (vs dasatinib alone) - ↑ Cell cycle arrest (sub-G1) - ↓ Cell cycle progression (S and G2/M)	[20]
Acute lymphoblastic leukaemia (ALL)	*In vitro:* Human cell lines - ALL: CCRF-CEM) - Burkitt's lymphoma: Ramos (PTK7 negative control)	**s-TDN:DOX** - Sgc8 aptamer - *Conjugate*: DNA tetrahedral nanostructure - *Cargo*: doxorubicin	*In vitro:* Application in the culture medium	*In vitro*: - ↑ Drug specificity - ↓ Growth of PTK7$^+$ cells - ↓ Cytotoxicity against PTK7$^-$ cells	[21]
Cancers with and without PTK7 expression	*In vitro*: Human cell lines - Breast adenocarcinoma: MCF-7 - Colon carcinoma: HCT-116 - Burkitt's lymphoma: Ramos (PTK7 negative control) *In vivo:* - s.c. xenograft mouse model	**Sgc8-NFs-Fc/Dox** - Sgc8 aptamer - *Conjugate*: DNA nanoflowers + ferrocene - ` doxorubicin	*In vitro*: Application in the culture medium *In vivo*: Intratumoral injection, every second day (0.5 mg/kg for the first week and 1 mg/kg for the last 2 weeks)	*In vitro*: - Good stability - Release of Dox proportional to H_2O_2 content (tumor cells) - Good specificity to PTK7$^+$ cells *In vivo:* - Stable in plasma - ↑ Antitumor capacity (vs other variants), without side effects	[22]

Table 1. Continued

Cancer type	Models	Approach for specific targeting	Therapeutic modalities	Impact of PTK7 targeting	Ref
Acute lymphoblastic leukaemia (ALL)	*In vitro*: Human cell lines - ALL: CCRF-CEM - Burkitt's lymphoma: Ramos (PTK7 negative control)	**Dox:Sgc8/hp-Au NP** - Sgc8 aptamer - *Conjugate*: hairpin DNA–gold nanoparticle (hp-Au NP) - *Cargo*: doxorubicin	*In vitro*: Application in the culture medium + laser irradiation	*In vitro*: - ↓ Cytotoxicity in non-tumor cells - ↑ Antitumor efficacy with phototherapy - Spatial/temporal control of Dox release by laser irradiation - Constructed nanoconjugates adapt to high drug load	[23]
Acute lymphoblastic leukaemia (ALL)	*In vitro*: Human cell lines - ALL: CCRF-CEM) - Burkitt's lymphoma: Ramos (PTK7 negative control)	**ASO/DOX/MB@PA/PDN** - Sgc8 aptamer - *Conjugate*: Hydrophobic polydopamine (PDA) coated DNA nanoballs - *Cargo(s)*: * Antisense oligonucleotides (ASO): Dz13, OGX-427 * Doxorubicin (Dox) * Methylene blue (MB)	*In vitro*: Application in the culture medium + Light irradiation for photodynamic or photothermal therapy	*In vitro*: - No cytotoxicity of PA/PDN (without light irradiation) - For each strategy: ↓ cell viability in PTK7$^+$ cells - Synergistic effect of multiple therapies	[24]
Colon cancer (CC)	*In vitro*: Human cell line - CC: HCT-116 *In vivo*: - S.c. xenograft mouse model	**EB-Sgc8** - Sgc8 aptamer - *Conjugate*: Evans blue (EB)	*In vitro:* Application in the culture medium *In vivo*: Intravenous injection	*In vitro*: - Improved stability - No toxic effect on PTK7- cells *In vivo:* - ↑ Tumor accumulation (vs Sgc8 alone) - ↓ Clearance of EB-Sgc8 by liver and kidneys (vs Sgc8 alone) - No toxic effect on normal tissues	[25]
Cancers with and without PTK7 expression	*In vitro:* Human cell lines - Cervical adenocarcinoma: HeLa - Breast adenocarcinoma: MCF-7: - Hepatoma: HepG2 (PTK7 negative control) - Normal liver cells: LO2 (PTK7 negative control)	- Sgc8 aptamer - *Conjugate*: pyropheophorbide	*In vitro*: Application in the culture medium + laser irradiation	*In vitro*: - Specific recognition of PTK7$^+$ tumor cells - ↓ Cell viability in PTK7$^+$ cells - No change in cell viability in the dark, w/o laser induction - Increased pyropheophorbide solubility	[26]

Table 1. Continued

Cancer type	Models	Approach for specific targeting	Therapeutic modalities	Impact of PTK7 targeting	Ref
Cancers with and without PTK7 expression	*In vitro*: Human cell lines - Cervical adenocarcinoma: HeLa - Burkitt's lymphoma: Ramos (PTK7 negative control) - Normal liver cells: LO2 (PTK7 negative control) *In vivo*: - S.c. xenograft mouse model	**UAS-PD** - Sgc8 aptamer - *Conjugate(s)*: * upconversion nanophosphor (UCNP) * pyropheophorbide a (PPA) - *Cargo:* Doxorubucin	*In vitro*: Application in the culture medium + laser irradiation *In vivo*: Intravenous injection + laser irradiation	*In vitro:* - Specificity for PTK7 + cancer cells - No obvious side effects on non-cancerous cells - ↑ Cell permeability for DOX (laser-enhanced). - ↑ Cancer cell killing (vs UAS-P, wo Dox) - Blockade of drug efflux pathway. *In vivo*: - Highly selective for target cancer cells - ↑ Retention in tumors - No obvious side effects on non-cancerous cells - ↑ Tumor tissue damage (laser-induced) - ↓ Tumor volume (laser-induced)	[27]
Inhibitors					
Atypical Teratoid Rhabdoid Tumors (ATRT)	*In vitro*: Human cell lines - ATRT: CHLA-05-ATRT, CHLA-06-ATRT	**Vatalanib**	*In vitro*: Application in the culture medium	- Inhibition of PTK7 expression - Cytotoxicity	[28]
Colon cancer (CC)	*In vitro*: Human cell lines - Colon carcinoma: HCT116, SW480	**01065, 03653, 20279** Inhibitors of PTK7/B-catenin interaction	*In vitro*: Application in the culture medium	- Modulation of Wnt pathway gene expression - ↓ Anchorage-independent cell growth - ↓ Cell viability - ↑ Cell cycle arrest	[29]

2 months, and a similar regression was observed in an SCC PDX model for 1 month.

Altogether, these 2 ADCs alone but also combined with other treatments demonstrate promising results in targeted therapy of PTK7-expressing solid tumors which warrant their further development towards a larger clinical application.

CAR-T cells

CAR-T cells were developed in the late 1980's by Eshhar and collaborators who have been exploring ways to enhance the immune system's ability to target and destroy cancer cells.[33] Since then, 6 CAR products have been FDA-approved and are currently available on the market for blood cancer therapy.[34] The CAR is a modified T-cell receptor created in the laboratory, allowing cells to recognize an antigen of interest. It is composed of 3 domains: (1) an antigen-binding domain which is usually based on a single-chain variable fragment (scFv) of a monoclonal antibody that allows it to recognize specifically cancer cells, (2) a co-stimulatory domain that permits the multiplication and persistence of CAR-T cells in the body, and (3) a signaling domain with anticancer activity. CAR-T cells are developed from the patient T-cells that are genetically modified to express the CAR. These CAR-T cells are amplified and infused back into the patient, for the specific recognition of a protein at the surface of cancer cells.[35]

Currently available CAR-T cell therapies are directed against CD19 and B-cell maturation antigen (BCMA), respectively for patients with B-cell malignancies[36] and relapsing/refractory multiple myeloma.[37] However so far, the therapeutic potential of CAR-T cells for solid tumors remains elusive, which endorses further efforts. In this context, the development of CAR-T cells targeting PTK7 could therefore be an interesting approach, and a couple of preclinical studies have shown encouraging results. Lee et al. have developed PTK7 CAR-T cells based on a scFv of PTK7-directed antibody (Patent US9102738B2). These PTK7 CAR-T cells induced significant cell death in PTK7-positive neuroblastoma (NB) cells and PDX models. Mock T cells or CD19 CAR-T cells had no significant cytotoxicity effect, and PTK7 knock-out NB cells were not sensitive to this therapy.[9]

A similar effect was found in OVCA, NSCLC, SCLC, breast, and pancreatic cancer cells confronted to CAR-T cells modified with a scFv derived from the hu6M024 sequence (cofetuzumab). Indeed, 2 research teams found a high level of production of IFN-γ and IL-2 by these CAR-T cells, directly proportional to the level of PTK7, as is the level of CAR-T cell proliferation.[16,17] Interestingly, in the study of Jie et al, the presence of PTK7-CAR-T cells increases the level of granzyme B and decreases PD-1, which confers a better efficacy of the treatment.[16] Altogether, these results demonstrate that PTK7 CAR-T cells are specifically cytotoxic on PTK7-expressing tumor cells, decrease tumor growth and prolong overall survival.

Aptamers

Aptamers are oligomers of synthetic single-stranded DNA or RNA, generated from large synthetic libraries of random oligonucleotide sequences, via an in vitro selection technologies, the most used one known as "systematic evolution of ligands by exponential enrichment" or SELEX.[38] Their specific oligomeric architecture confers them peculiar binding capacities to multiple targets, that is, ions, small molecules, proteins, microorganisms, or cells. Like antibodies, aptamers can act on extracellular targets, but are smaller, and can be chemically synthetized in cell-culture free systems. They can serve multiple purposes: (1) they can be used for biomarker detection, (2) they can act as antagonists on their targets and inhibit molecular interactions, or (3) they can deliver cargo molecules upon internalization by a cell, for example, chemotherapy. Finally, aptamers can be chemically modified for improved pharmacokinetics.[39] To date, 2 RNA aptamers are approved by the FDA. Pegaptanib and acincaptad pegol respectively target the vascular endothelial growth factor (VEGF) and complement protein C5, and are used for the treatment of age-related macular degeneration and secondary geographic atrophy.[40] Whereas no aptamer has yet entered cancer therapy pipelines, many are under investigation for targeting soluble factors or cell surface proteins within tumors.[41] In this review, we list evidence suggesting or demonstrating the therapeutic potential of aptamers targeting PTK7.

Sgc8 is the first PTK7-specific aptamer that was developed by Shangguan et al using cell-based selection. It has below nanomolar affinity for the target and was shown to specifically recognize leukemia cells among other healthy bone marrow components.[42,43] Taghidisi et al used Sgc8 coupled with daunorubicin (Dau:Sgc8) and demonstrated efficient and specific internalization of Sgc8-Dau in PTK7 + ALL cells, however not significantly better than Dau alone, but showed a decreased cytotoxicity in PTK7-cells which indicates reduced side effects.[18] Xiang et al used this PTK7-targeted, Sgc8 aptamer for the specific delivery of gemcitabine (GEM) to PTK7-expressing bladder cancer (BC) cells. Sgc8-GEM demonstrated a good safety profile in vivo, inhibited tumor proliferation, and induced higher tumor-specific cytotoxicity compared to GEM treatment alone. The Sgc8-GEM-treated mice also showed reduced amount of lung metastases.[19] In the same line, Sicco et al coupled PTK7-targeted Sgc-8 aptamer with dasatinib (Sgc8-c-carb-da) for which they demonstrated better cytotoxicity than dasatinib alone, based on the detection of cell cycle arrest, late apoptosis and necrosis in mouse and human lymphoma cells.[20]

Several studies have also used the Sgc8 aptamer together with the widely used anthracycline antitumor molecule doxorubicin, in a wide variety of therapeutic combinations. Liu et al used the Sgc8 to modify a tetrahedral DNA nanostructure (TDN), described as a large, stable, and biocompatible drug delivery system that could be loaded with multiple compounds,[44] for example, chemotherapeutic molecules. The Sgc8-TDN (s-TDN) loaded with doxorubicin (s-TDN:DOX) was validated in vitro on lymphoblastic leukemia cells, and was shown to specifically inhibit PTK7-positive cell growth and reduce cytotoxicity against PTK7-negative cells.[21] In a recent study, Zhang et al coupled the Sgc8 aptamer to DNA nanoflower-based biosensors[45] with a ferrocene group (Fc) and conjugated to doxorubicin, aiming at a controlled degradation of the structure upon cell internalization, for a fine-tuned release of doxorubicin in the targeted tumors. They demonstrate that Sgc8-NFs-Fc/Dox has a good stability and specificity for targeting PTK7$^+$ cells, reducing Dox cytotoxicity on non-target cells. They also showed that Dox release is proportional to the presence of H_2O_2, present intracellularly in tumor cells. In vivo validation included subcutaneous transplantation of colon carcinoma cells with intratumoral injections of Sgc8-NFs-Fc/Dox, which showed higher antitumoral activity compared to different controls.[22] Finally, the combination of doxorubicin-based chemotherapy with

phototherapy was studied by Luo et al who developed Sgc8-directed hairpin DNA-gold nanoparticles, conjugated with doxorubicin (Dox:Sgc8/hp-Au NP). They showed that their nanoconjugate structure could deliver a large drug amount to target cells, in a PTK7-selective manner, with reduced side effects in negative cells. Of note, cell illumination with laser-controlled, time and space-resolved plasmon resonance light therapy (532 nm) induced Dox release and improved efficacy.[23] Phototherapy was also used as a sole therapeutic approach, with aptamer-based targeting. Xiong et al conjugated the Sgc8 aptamer to the photosensitizer molecule pyropheophorbide. They showed a dose-dependent decrease in viability of PTK7-positive cancer cells upon treatment, whereas cell viability remained above 95% for PTK7-cells, or when cancer cells were incubated in the dark.[26] Another study developed a combination of photosensitization and doxorubicin release. Jin X et al worked on the conjugation of Sgc8-directed, light-activable theranostic nanoformulation based on pyropheophorbide for cancer-targeted doxorubicin release. They showed that their structure has high PTK7 specificity, good tumor retention, hence no obvious side effects on non-cancerous cells. In addition, they demonstrated a blockade of drug efflux pathways (efflux transporter, P-gp protein).[27]

Aiming at the optimization of a more durable, safer PTK7-targeting structure before attaching any therapeutic agent, Ding et al examined the possibility of modifying Sgc8 with the small molecule Evans Blue (EB) to enable albumin binding for prolonged maintenance in the blood flow. They used mouse xenograft models as well as in vitro models to illustrate the improved stability of the aptamer. They demonstrated better and prolonged accumulation of the EB-Sgc8 aptamer compared with the Sgc8 aptamer, and the EB-Sgc8 systemic clearance was also delayed compared with Sgc8, with no toxic effect on normal tissues.[25]

To propose non-covalent binding strategies of aptamers to DNA-based nanomaterials, and make them a more versatile delivery tool, Shim et al used polydopamine for the coating of DNA nanoballs (PDN) to attach the Sgc8 aptamer (which they named PA) via hydrophobic interactions. Shim et al then proposed diverse therapeutic strategies based on these PA/PDN structures. They conjugated these PTK7-targeted PA/PDN with (1) previously described antisense oligonucleotides for the silencing of Jun and HSP27 genes; (2) the photosensitizer methylene blue, for photodynamic therapy; or (3) doxorubicin for chemotherapeutic effect. Finally, they also proposed this structure for a photothermal effect induced after heating of the polydopamine coating. In vitro, each of these 4 strategies showed efficiency against PTK7+ cancer cells, reducing their viability, while sparing PTK7-negative cells. The results also demonstrated a potential multimodal therapeutic strategy, combining photodynamic, photothermal and gene-silencing effects.[24]

Other experimental approaches for PTK7 inhibition

The abnormal activation of RTKs and downstream pathways, due to epigenetic or genetic alterations, can lead to uncontrolled cell growth, prolonged tumor maintenance, and propagation. In this context, a broad panel of tyrosine kinase inhibitors have been developed, FDA-approved, and used for cancer treatment. To date, there is no small molecule used for the inhibition of PTK7 signaling. However, recent studies have proposed putative candidates. Messerli et al used valatinib, a broad-spectrum tyrosine kinase inhibitor, and demonstrated cytotoxicity against atypical teratoid rhabdoid tumors cultures. They showed that valatinib decreases PTK7 expression. In parallel, they showed that PTK7 knockdown via small interfering RNA reduces cell viability. Whether valatinib has any direct effect on PTK7, or rather indirectly modulates PTK7 signaling through inhibition of other RTKs (eg, KDR), remains unanswered.[28]

In a recent study, Ganier et al developed inhibitors targeting the Wnt signaling pathway and more precisely, the PTK7/β-catenin interaction thanks to a high-throughput NanoBRET screening assay in living colon carcinoma cancer cells. Seven inhibitors were identified with micromolar range potency and good selectivity and 3 inhibitors (01065, 03653, and 20279) were further tested. Treatment of cancer cells with those inhibitors induced changes in Wnt-related target genes, decrease in cell viability, cell cycle arrest, and increased apoptosis.[29] Whether these effects antitumor effects are mechanistically dependent on the inhibition of PTK7/β-catenin interaction remains to be confirmed, for example, using PTK7-negative cells.

Conclusions and perspectives

PTK7 has emerged as a potential therapeutic target for human tumors, and the last years of research have consolidated 3 prominent therapeutic approaches. Among ADCs, cofetuzumab pelidotin demonstrated antitumor effects in vitro and in various human cancer models with a relatively good safety profile, which has then launched phase I studies that resulted in notable responses in patients with breast, ovarian, and lung cancer.[12] The safety and efficacy of this ADC in recurrent NSCLC patients are under validation in an ongoing phase I trial for patients with recurrent NSCLC (NCT04189614). Another ADC (ie, MTX13) was recently introduced and demonstrated good preclinical safety and efficacy, with wide therapeutic index,[15] and will likely progress toward clinical validation. Preliminary information reveals the development of a novel bispecific ADC, targeting PTK7 together with the TROP2 receptor[46] that is highly expressed by breast cancer cells and already targeted using another ADC.[47] This indicates that anti-PTK7 ADC research is a continuously evolving field that likely will bring further insight into cancer targeted therapy. Additionally, some studies suggest that whereas ADCs alone may not result in sufficient therapeutic responses, they demonstrate clinical benefits when used in combination with other treatments, for example, chemotherapies.

Anti-PTK7 CAR-T cells are also under intensive development. Preclinical studies indicate that they induce significant tumor cell death in different cancer types, as well as the production of proinflammatory cytokines and modulation of the immune response. Like ADCs, CAR-T cell therapy yet faces several key challenges, including off-tumor effects, side toxicity or tissue penetration,[35] still anti-PTK7 targeting in cancer using this approach warrants further development.

Aptamers constitute a third therapeutic approach for PTK7 targeting. These molecules have a subnanomolar affinity for their target, allowing precise identification of tumor cells. Anti-PTK7 aptamers have been used in a variety of ways, including in combination with chemotherapeutic drugs, and prove higher antitumor effects compared to the drug alone. Furthermore, researchers are developing increasingly

intricate nanostructures and biosensors that, when linked to PTK7-aptamers, allow for greater and more specific anti-tumor effects. Phototherapy is also employed in various studies to regulate the timing of drug release and enhance the efficacy and specificity of drugs towards the target cancer cells. The use of aptamers is positioned as the therapy with the greatest modulation potential. Interestingly, aptamers also have been suggested as tools for refining CAR-T cell therapies, for example, by improving CAR-T cell production, reducing side toxicity, or even enable cell tracking in vivo.[48] Interestingly, the Sgc8 aptamer coupled to gallium is under clinical investigation as a tool for refined staging of bladder cancer (NCT06005116), but it remains to be seen how PTK7-aptamers will translate towards the clinics for cancer therapy.

In summary, the multifaceted landscape of PTK7-targeted therapies in cancer underscores the potential of these innovative approaches to revolutionize cancer treatment paradigms. By exploiting the aberrant expression of PTK7 in solid tumors, these therapies offer a targeted and personalized approach to combatting cancer with enhanced precision and efficacy.

Author contributions

Conception of the study: Bernard Rogister and Virginie Neirinckx. Writing—original draft preparation: Kim Mottard, Julie Cokaiko, Virginie Neirinckx. Writing—review and editing: Kim Mottard, Julie Cokaiko, Bernard Rogister, Virginie Neirinckx. Supervision: Virginie Neirinckx. Funding acquisition: Bernard Rogister BR and Virginie Neirinckx. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

No new data were generated or analyzed in support of this research.

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