UJEMI PEARLS

Oncolytic Viruses, Bi-specific T Cell Engagers and CAR-T Cells: A Promising Triple Combination Therapy for Pancreatic Ductal Adenocarcinomas

Gillian Savage

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Pancreatic ductal adenocarcinoma (PDAC) is a lethal malignant cancer of the pancreas, with less than 5 percent of patients surviving five years after diagnosis. Due to the lack of early symptoms, initial diagnosis is often difficult, leading to a metastatic disease that can no longer be cured by surgical removal. Alongside this, the conventional methods of cancer therapies, such as chemotherapy and radiotherapy, are not effective for PDACs. However, in recent years, three novel therapeutic agents for treatment of cancer have emerged: oncolytic viruses (OVs), bi-specific T-cell engagers (BiTEs), and chimeric antigen receptor (CAR) T cells. Oncolytic virus treatments involve the use of viral replication cycles and viral tropism to target cancerous cells, and selectively kill them through lysis. Meanwhile, BiTEs allow the cytotoxic killing of cancer cells, including those that lack MHC expression, by linking tumor cells and cytotoxic T cells through bi-specific monoclonal antibodies. CAR-T cell immunotherapy utilizes the immune system to kill cancer cells by genetically modifying T cells to produce receptors targeted at specific cancer antigens. While all these therapies have had successes individually, problems have been associated with all of them for pancreatic cancer treatment. Recently, research has shown that oncolytic viruses can be engineered to encode and express BiTEs, targeting their expression to the cancerous cells. Combining this with CAR-T therapy provides a robust treatment with great therapeutic potential. This article will explore the use of oncolytic viruses, BiTEs and CAR-T cells as a treatment for PDACs, including the strengths and limitations of these treatments separately and as a combination therapy. Understanding how to combine these three therapeutic agents and target them to pancreatic cancer cells could result in a novel cancer therapy for PDACs, a cancer that currently does not have many promising treatments.

INTRODUCTION

In Canada, pancreatic cancer is the fourth leading cause of cancer-related death, and the majority of pancreatic malignancies are pancreatic ductal adenocarcinomas (PDAC), exocrine tumors that develop from pancreatic duct cells [1–3]. PDAC is difficult to diagnose, with a lack of early symptoms and no standardized screening tests, resulting in late diagnosis [2]. Treatment also remains difficult for PDAC, with the only curative treatment being complete removal through invasive surgery [1]. However, with the lack of early diagnosis, surgery is often not an option as the cancer has already reached metastasis. At this stage, chemotherapy and radiation are ineffective and attempted immunotherapies yet to have successful outcomes [4].

With recent progression in immunotherapy, three new novel therapeutic agents for cancer treatment have evolved: oncolytic viruses (OVs), bi-specific T cell engagers (BiTEs), and chimeric antigen receptor (CAR) T cells. OVs are viruses that have been modified to target and replicate within cancer tumor cells, inducing death through cell lysis [5]. These viruses are attenuated to reduce virulence and not cause disease within the host while still allowing for the host to amount an immune response. Some viruses are chosen because they naturally target the cancerous cell while others are genetically modified to

Published Online: September 2020

Citation: Gillian Savage. 2019. Oncolytic Viruses, Bi-specific T Cell Engagers and CAR-T Cells: A Promising Triple Combination Therapy for Pancreatic Ductal Adenocarcinomas. UJEMI PEARLS 4:1-8.

Editor: François Jean (Ph.D.), University of British Columbia

Address correspondence to Gillian Savage

gilliansav@gmail.com

target tumor antigens [6]. The lytic activity of the virus also induces inflammation within the tumor microenvironment (TME) causing an influx of immune cells, which promotes an immune response against the tumor cells [7].

BiTEs are engineered proteins consisting of two single-chain variant fragments, the antigen-binding segments of an antibody, joined by a linker, with one chain recognizing a tumor-associated antigen and the other recognizing CD3 [8]. Binding of several BiTEs to the tumor-associated antigens on the tumor cell and CD3 on host T cells causes clustering of CD3, leading to downstream T cell signaling and formation of a synapse between the two cells. The downstream signaling within the T cell triggers a targeted release of cytotoxic granules into the tumor cell, leading to tumor cell apoptosis [9,10]. Therefore, this therapy provides an effective cancer cell treatment through targeting the host's cytotoxic T cells to kill cells expressing the appropriate tumor antigen.

CAR-T cell therapy consists of isolating T cells from a patient and engineering them to express a chimeric antigen receptor (CAR), a modified T cell receptor that specifically recognizes a tumor-associated antigen [11]. These cells are then infused back into the patient's body, where they are directed towards tumor cells through recognition of the anti-CAR antigen, leading to induction of the CAR-T cell's cytotoxic killing activity [11]. OVs, BiTEs, and CAR-T cells all show remarkable promise for tumor treatment but still face limitations on their own. Therefore, there may be difficulties in using these therapeutics to treat the aggressive, immunoresistant PDAC. However, it has been found that BiTEs can be engineered into oncolytic viruses and combined with CAR-T cells to create a therapy effective at clearing solid tumors [12]. This paper proposes that a combination of these three therapies has the potential to effectively treat and clear PDACs.

RESEARCH QUESTIONS

Due to the lack of early symptoms, PDAC is often diagnosed once it is already metastatic and the only curative treatment, surgical removal, is no longer an option [1]. At this stage, there are no effective therapies for treating PDAC [2]. It has been found that BiTEs can be engineered into the genome of OVs and combined with CAR-T cells to create a therapy effective at clearing solid tumors [5]. This paper proposes a combination of these three novel therapies and their combined potential to effectively treat and clear PDACs. First, reasons that OVs, BiTEs, and CAR-T cell therapies are not effective for PDAC on their own will be discussed. Second, the potential of the combination of these therapies for PDAC treatment will be explored. Finally, this paper will address the challenges associated with this combination therapy. Altogether, this paper intends to explore how to combine OVs, BiTEs and CAR-T cells and target them to pancreatic cancer cells in order to develop a novel cancer therapy for PDACs.

PROJECT NARRATIVE

Why are oncolytic viruses, bi-specific T-cell engagers and chimeric antigen receptor T cells not effective PDAC treatments on their own? In recent years, various experiments and trials have been conducted using oncolytic viruses, BiTEs, and CAR-T cells as a treatment for pancreatic tumors. However, complete tumor regression has rarely been seen when each of these therapies has been used alone. ONYX-015, an oncolytic virus that infects cells that have a mutation in p53, went through a phase I trial for treating pancreatic carcinomas [13,14]. While the treatment was well tolerated by the patients, there was no response seen, and the tumor cells showed resistance to viral replication [14,15]. However, the use of OVs on cancers provides some significant advantages besides tumor-cell death through lysis. The lytic activity of OVs also induces an anti-tumor response, causing an influx of T cells into the TME, as well as the release of cancerous antigens that can be utilized by immune cells to further mount an adaptive immune response [7]. OVs can also be engineered to express other therapeutics, such as cytokines and monoclonal antibodies[5]. Despites these benefits, there are still some problems to overcome for OV therapy. One major issue of OVs is their premature clearance as a result of the host's immune response against them [7]. Another issue is that in PDAC tumors, the stromal cells

can sometimes compose up to ninety percent of the tumor volume [16]. This creates a dense stromal barrier, making the odds of an OV diffusing into the TME low [17].

A BiTE that recognizes tyrosine kinase orphan receptor 1 (ROR1) which is found in high abundance on pancreatic cancer cells, has been used for treatment against pancreatic cancer [18,19]. The ROR1-BiTE was able to successfully induce T cell killing of pancreatic tumor cells in xenograft models, causing tumor reduction, but did not result in a complete cure [18]. BiTEs provide great therapeutic potential for pancreatic cancer due to their ability to direct CD8+ T cell-mediated killing of tumor cells independently of expression levels of MHC molecules, which are normally downregulated by cancer cells [9]. However, BiTEs are unable to discriminate between tumor and normal levels of the target antigen, resulting in BiTE-mediated killing of healthy cells [20]. PDAC shows resistance to BiTE therapy due to its highly immunosuppressive TME, contributing to a lack of T cells available for BiTE-mediated killing [1]. Another major issue of BiTEs is their short serum half-life, causing a need for constant infusions, resulting in increased toxicity and expenses [12].

CAR-T cell therapy against PDAC has gone through a Phase I trial using a CAR that recognizes mesothelin, a cell surface glycoprotein normally overexpressed on PDAC cells [21]. While the treatment caused stabilization in 2 of the 6 patients, this lasted for less than 6 months, and was then followed by tumor regression [21,22]. Similar to BiTEs, CAR-T cells also show promise as a treatment for PDAC due to their ability to target T cells to specifically kill tumor cells even in the absence of MHC expression [9]. Their long-term persistence and relatively short treatment time are also appealing aspects, but limitations still exist [23]. The immunosuppressive TME prevents homing of the introduced CAR-T cells to the tumor-antigen expressing cells [1]. One of the major problems with CAR-T cell therapy is the ability of the tumor cells to downregulate the antigen the CAR-T cell recognizes. Known as tumor escape, this mechanism helps the tumors hide from the CAR-T cells and escape their cytotoxic killing [23].

Therapy	What is being used for PDAC	Advantages	Problems/Limitations
Oncolytic viruses	• ONYX-015	• Tumor-cell lysis • Induce anti-tumor immune response • Can engineer to encode therapies	• Clearance by host's immune system • Acute toxicities • Stromal barrier of PDAC
Bi-specific T cell engagers	• ROR1 BiTE	• Works independently of MHC expression • Directs CTLs to kill tumor cells	• Unable to discriminate between tumor & normal levels of antigen • Low # of T cells in TME • Poor delivery, short ½ life
CAR-T cells	• CAR-Tmeso (Phase I)	• Works independently of MHC expression • Engineered to target cancer specific protein • Short treatment time • Long-term persistence	• Limited efficiencies • Immunosuppressive TME • Tumor escape

FIG. 1 Advantages and limitations of oncolytic viruses, bi-specific T cell engagers, and chimeric antigen receptor-T cells for treating pancreatic ductal adenocarcinomas. Oncolytic viruses, bi-specific T cell engagers and chimeric antigen receptor (CAR)-T cells have all undergone testing as treatment for pancreatic ductal adenocarcinomas (PDAC) on their own. While all of these have advantages, these therapies experience limitations because of the difficult and resistant characteristics of PDAC. ONYX-015 is an oncolytic virus that targets cells with a mutation in p53 [13]. ROR1 BiTE targets tyrosine kinase orphan receptor 1, which is highly expressed on pancreatic cancer cells [18,19]. CAR-Tmeso targets mesothelin, another overexpressed protein on PDAC cells [21,22]. While all of these have been used to treat PDAC, none have shown any promising results.

How would the combination of these techniques result in better treatment of PDACs? One great benefit of OVs is their ability to be engineered to contain other therapeutics [5]. Therefore, OVs can be engineered to express BiTEs that recognize an antigen overexpressed on PDAC cells by inserting the gene for the PDAC-specific BiTE into the OV's genome [5]. This can then be combined with CAR-T cells that recognize another PDAC antigen for a highly effective triple combination therapy (illustrated in Figure 2) that counteracts the limitations of each therapeutic approach on their own [12].

OVs cause inflammation with their lytic activity, disrupting the immunosuppressive defense of the cancer and causing an influx of immune cells to the TME, including T cells that can then be utilized by BiTEs [5,24]. The CAR-T cells that normally have difficulty reaching the immunosuppressive TME are now able to home to the TME because of this increased immune cell infiltration generated by OVs [12]. By engineering the OV to express BiTEs, BiTE expression is limited to the TME, preventing BiTE-mediated killing of healthy tissue [5]. This also allows for a high concentration of BiTEs in the TME, improving efficiencies and removing the need for constant infusions, since BiTEs will continue to be expressed as long as the OV is replicating [5].

BiTEs in the TME synergize with OVs to improve viral spread by aiding in the reduction of tumor density and are also capable of redirecting anti-viral CD8+ T cells towards tumor cells expressing the BiTE antigen, helping prevent the premature clearance of the OV since the CD8+ T cells are now directed towards virus-free tumor cells rather than just the virally infected cells [5]. BiTEs also allow the CAR-T cell to induce cytotoxic killing of tumor cells through two antigens, by redirecting the CAR-T cell towards a secondary tumor antigen that the BiTE recognizes in addition to what the CAR recognizes. This allows CAR-T cells to be effective killing agents even if tumor escape occurs [12].

The combination of CAR-T cells with BiTE-expressing OVs has a synergistic effect on tumor cell death [12]. In fact, Wing et al found that the combination of all three therapeutic agents was the only treatment that led to pancreatic tumor regression in a xenograft mouse model [12].

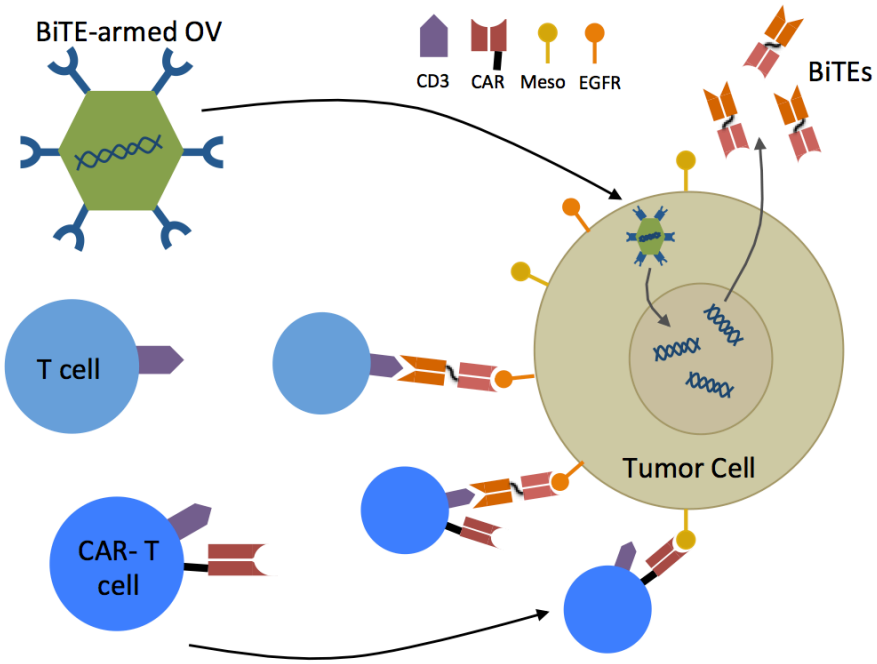

FIG. 2 Bi-specific T cell engager (BiTE)-armed oncolytic viruses (OVs) are programmed to specifically target and infect tumor cells. Once infection occurs, the viruses begin to replicate, producing more OVs as well as BiTEs, which are then released into the tumor microenvironment with tumor cell lysis. BiTES can then redirect either the patient's T cells or the introduced chimeric antigen receptor (CAR)-T cells towards the cell expressing the appropriate tumor antigen recognized by the BiTE. CAR-T cells can also kill tumor cells through recognition of a second tumor antigen that the CAR recognizes. In this example the BiTE recognizes epidermal growth factor (EGFR), and the CAR recognizes Mesothelin (Meso), which are both commonly overexpressed in PDAC [38,39].

What are the challenges associated with this combination therapy? While this triple combination therapy has great promise, there are still some challenges associated with it. First, the cost of such a therapy is a major barrier, reducing the number of people to whom it will be available. The FDA-approved T-VEC, an OV for treatment of melanomas, costs $65,000 USD [25]. Blinatumomab, an FDA-approved BiTE therapy for B cell acute lymphoblastic leukemia, costs around $178,000 USD per year [26,27]. CAR-T cell therapy however, is the most expensive, with the FDA-approved Tisagenlecleucel, also for treatment of B cell acute lymphoblastic leukemia, costing $475,000 USD [28,29]. With these current prices, it is reasonable to assume that this triple combination therapy will cost around $650,000 USD. However, this triple combination therapy proposes to engineer the BiTEs within the OV, causing constant production of them as long as the virus is still replicating. This removes the need for constant infusions of the BiTE and eliminates the ongoing cost of administering BiTE therapy, which is a major expense of BiTE therapy. Therefore, with this proposed therapy, the cost of BiTEs could be rolled into the cost of the OV, bringing down the overall estimated cost to around $500,000 USD. While this cost is significantly lower, this is still a highly expensive therapy. However, the lack of effective treatments for PDAC makes this treatment worth investing into despite the high cost. Cost reduction for this therapy is also possible since CAR-T cell and OV therapies are still relatively new and with time it is expected they should go down in price as technologies improve and become more available on the market [29].

Another major issue is the physical properties of PDAC, including necrosis, hypoxia, acidosis and the high abundance of stromal cells [16,30,31]. These factors disrupt OV spread and replication and it is important to address how these problems can be overcome [7]. The administration of OVs and CAR-T cells for maximum effectiveness must also be addressed. Overall, it would be best to administer the BiTE-expressing OV first to induce inflammation and then follow this with CAR-T cell administration into the bloodstream [23]. The inflammation induced by initial OV administration would allow the CAR-T cells to infiltrate the TME. Consideration needs to be taken as to how the OVs should be administered. Systematic injection is beneficial for metastatic PDAC, however the dense stromal barrier of the PDAC might make it difficult for the systematically administered

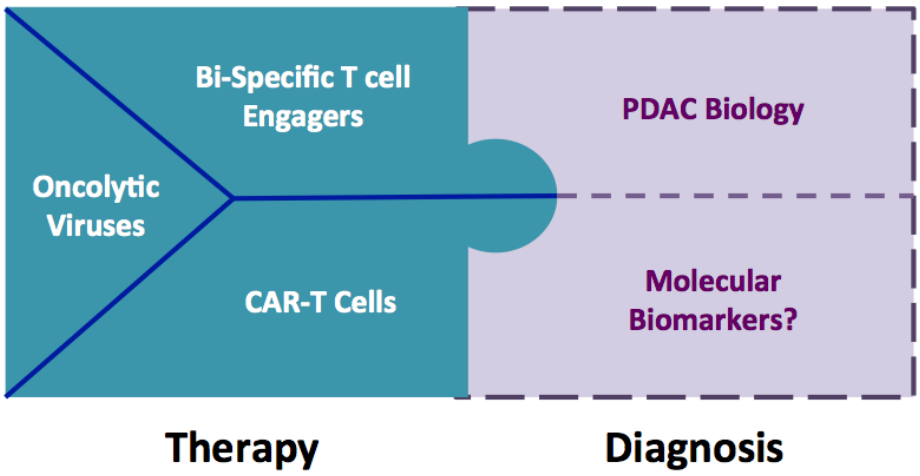

FIG. 3 Schematic of key pieces of a successful treatment of pancreatic ductal adenocarcinomas. This paper proposes the use of a novel therapy for pancreatic ductal adenocarcinomas (PDAC) involving three key components: oncolytic viruses, bi-specific T cell engagers, and chimeric antigen receptor (CAR)-T cells. However, lack of effective diagnostic tools for PDAC makes this therapy incomplete. Research on proper diagnosis for PDAC is the next step and is crucial for the success of this treatment. Diagnostic research should incorporate the biology of PDAC and the possibility of using molecular biomarkers.

OVs to infect the tumor cells [16,23]. Injection of the OVs directly into the tumor might be best for PDACs since it would bypass the stromal barrier and insert the OVs directly into the TME [32]. However, this may not be advantageous if the disease has already reached metastasis. Therefore, for the best result, it would be logical to propose the administration of the BiTE-expressing OVs through both systemic and tumor-specific injection.

CONCLUSIONS

PDAC is the most common malignancy of the pancreas and is the fourth leading cause of cancer-related death, killing 4,800 Canadians per year [33]. Currently there is a lack of therapies that are effective at eliminating PDAC. Recently, oncolytic viruses have been an area of interest because of their potential as therapeutic agents, especially when combined with existing immunotherapies [7]. BiTEs and CAR-T cells are also promising immune-based therapeutics in the field of cancer therapy. However, all three of these therapies have limitations on their own, especially when treating PDAC, which provides many challenges due to its physical and immunosuppressive properties. This paper proposes a combination of these three therapies. By engineering OVs to express BiTEs and then combining this with CAR-T cells, this creates a highly robust therapy with potential to tackle PDAC. However, challenges such as cost and delivery still exist and need to be considered during development of this therapy.

In order for this approach to be successful, it is important to further research the biology of PDAC. A better understanding of the immunosuppressive and physical barriers of PDAC such as the dense stromal layer, necrosis, and hypoxia, and how to combat these would be beneficial in determining how to maximize viral spread and replication of the OV. In addition, one major difficulty of PDAC treatment is its late diagnosis [2]. Earlier diagnosis results in better treatment options being available, as well as a better likelihood that this proposed triple combination therapy would be successful.

Since current methods, such as CT scans and endoscopic ultrasounds, often diagnose patients too late, research into liquid biopsies and molecular diagnostics might prove to be beneficial for early diagnosis. Currently, CA19-9 is the best FDA-approved biomarker available used to detect PDAC [34]. However, its poor sensitivity and specificity makes it a poor marker for diagnosis of earlier stages [35]. Recently, exosome-associated miRNA-21 and miRNA-155 have been found to be present in high levels in the pancreatic juice of patients diagnosed with PDAC, and can be detected earlier than CA19-9 [36]. A new combination of biomarkers for thrombospondin-2 (THBS2), CA19-9, and circulating tumor DNA (ctDNA), has been found to diagnose PDAC in patients as early as stage I [37]. These are all relatively new diagnostics and have yet to reach clinics, but further research in this field might be the next important step for the success of this therapy.

Overall, understanding how to combine and target OVs, BiTEs and CAR-T cells towards pancreatic cancer cells could result in a novel cancer therapy for PDACs. Further, this research could open up a frontier on how the combination of OVs with immunotherapies has immense therapeutic potential for difficult to treat cancers.

ACKNOWLEDGEMENTS

I would like to thank Dr. François Jean for his guidance and recommendations during the development of this project. I would also like to thank my MICB406 classmates for their suggestions and critiques of my research questions and ideas during small group discussions.

REFERENCES

1. Foucher ED, Ghigo C, Chouaib S, Galon J, Iovanna J, Olive D. Pancreatic Ductal Adenocarcinoma: A Strong Imbalance of Good and Bad Immunological Cops in the Tumor Microenvironment. Front Immunol. 2018;9: 1044. doi:10.3389/fimmu.2018.01044
2. Ibrahim AM, Wang Y-H. Viro-immune therapy: A new strategy for treatment of pancreatic cancer. World J Gastroenterol. 2016;22: 748–763. doi:10.3748/wjg.v22.i2.748
3. Stanger BZ, Dor Y. Dissecting the Cellular Origins of Pancreatic Cancer. Cell Cycle. 2006;5: 43–46. doi:10.4161/cc.5.1.2291

4. Hilmi M, Bartholin L, Neuzillet C. Immune therapies in pancreatic ductal adenocarcinoma: Where are we now? World J Gastroenterol. 2018;24: 2137–2151. doi:10.3748/wjg.v24.i20.2137

5. Scott EM, Duffy MR, Freedman JD, Fisher KD, Seymour LW. Solid Tumor Immunotherapy with T Cell Engager-Armed Oncolytic Viruses. Macromol Biosci. 2018;18. doi:10.1002/mabi.201700187

6. Raja J, Ludwig JM, Gettinger SN, Schalper KA, Kim HS. Oncolytic virus immunotherapy: future prospects for oncology. Journal for ImmunoTherapy of Cancer. 2018;6: 140. doi:10.1186/s40425-018-0458-z

7. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov. 2015;14: 642–662. doi:10.1038/nrd4663

8. Baeuerle PA, Reinhardt C. Bispecific T-Cell Engaging Antibodies for Cancer Therapy. Cancer Res. 2009;69: 4941–4944. doi:10.1158/0008-5472.CAN-09-0547

9. Offner S, Hofmeister R, Romaniuk A, Kufer P, Baeuerle PA. Induction of regular cytolytic T cell synapses by bispecific single-chain antibody constructs on MHC class I-negative tumor cells. Molecular Immunology. 2006;43: 763–771. doi:10.1016/j.molimm.2005.03.007

10. Brischwein K, Parr L, Pflanz S, Volkland J, Lumsden J, Klinger M, et al. Strictly Target Cell-dependent Activation of T Cells by Bispecific Single-chain Antibody Constructs of the BiTE Class: Journal of Immunotherapy. 2007;30: 798–807. doi:10.1097/CJI.0b013e318156750c

11. CAR T Cells: Engineering Immune Cells to Treat Cancer. In: National Cancer Institute [Internet]. 6 Dec 2013 [cited 14 Feb 2019]. Available: https://www.cancer.gov/about-cancer/treatment/research/car-t-cells

12. Wing A, Fajardo CA, Posey AD, Shaw C, Da T, Young RM, et al. Improving CART-Cell Therapy of Solid Tumors with Oncolytic Virus–Driven Production of a Bispecific T-cell Engager. Cancer Immunol Res. 2018;6: 605–616. doi:10.1158/2326-6066.CIR-17-0314

13. Ries S, Korn WM. ONYX-015: mechanisms of action and clinical potential of a replication-selective adenovirus. Br J Cancer. 2002;86: 5–11. doi:10.1038/sj.bjc.6600006

14. Mulvihill S, Warren R, Venook A, Adler A, Randlev B, Heise C, et al. Safety and feasibility of injection with an E1B-55 kDa gene-deleted, replication-selective adenovirus (ONYX-015) into primary carcinomas of the pancreas: a phase I trial. Gene Therapy. 2001;8: 308–315. doi:10.1038/sj.gt.3301398

15. Hecht JR, Bedford R, Abbruzzese JL, Lahoti S, Reid TR, Soetikno RM, et al. A Phase I/II Trial of Intratumoral Endoscopic Ultrasound Injection of ONYX-015 with Intravenous Gemcitabine in Unresectable Pancreatic Carcinoma. Clin Cancer Res. 2003;9: 555–561.

16. Ying H, Dey P, Yao W, Kimmelman AC, Draetta GF, Maitra A, et al. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 2016;30: 355–385. doi:10.1101/gad.275776.115

17. Ady JW, Heffner J, Klein E, Fong Y. Oncolytic viral therapy for pancreatic cancer: current research and future directions. Oncolytic Virother. 2014;3: 35–46. doi:10.2147/OV.S53858

18. Gohil SH, Paredes-Moscosso SR, Harrasser M, Vezzalini M, Scarpa A, Morris E, et al. An ROR1 bi-specific T-cell engager provides effective targeting and cytotoxicity against a range of solid tumors. Oncoimmunology. 2017;6. doi:10.1080/2162402X.2017.1326437

19. Balakrishnan A, Goodpaster T, Randolph-Habecker J, Hoffstrom BG, Jalikis FG, Koch LK, et al. Analysis of ROR1 protein expression in human cancer and normal tissues. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23: 3061. doi:10.1158/1078-0432.CCR-16-2083

20. Stone JD, Aggen DH, Schietinger A, Schreiber H, Kranz DM. A sensitivity scale for targeting T cells with chimeric antigen receptors (CARs) and bispecific T-cell Engagers (BiTEs). OncoImmunology. 2012;1: 863–873. doi:10.4161/onci.20592

21. Beatty GL, O'Hara MH, Lacey SF, Torigian DA, Nazimuddin F, Chen F, et al. Activity of Mesothelin-Specific Chimeric Antigen Receptor T Cells Against Pancreatic Carcinoma Metastases in a Phase 1 Trial. Gastroenterology. 2018;155: 29–32. doi:10.1053/j.gastro.2018.03.029

22. Argani P, Iacobuzio-Donahue C, Ryu B, Rosty C, Goggins M, Wilentz RE, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). Clin Cancer Res. 2001;7: 3862–3868.

23. Guedan S, Alemany R. CAR-T Cells and Oncolytic Viruses: Joining Forces to Overcome the Solid Tumor Challenge. Front Immunol. 2018;9. doi:10.3389/fimmu.2018.02460

24. Guo ZS, Liu Z, Bartlett DL. Oncolytic Immunotherapy: Dying the Right Way is a Key to Eliciting Potent Antitumor Immunity. Front Oncol. 2014;4. doi:10.3389/fonc.2014.00074

25. Orloff M. Spotlight on talimogene laherparepvec for the treatment of melanoma lesions in the skin and lymph nodes. Oncolytic Virother. 2016;5: 91–98. doi:10.2147/OV.S99532

26. Przepiorka D, Ko C-W, Deisseroth A, Yancey CL, Candau-Chacon R, Chiu H-J, et al. FDA Approval: Blinatumomab. Clin Cancer Res. 2015;21: 4035–4039. doi:10.1158/1078-0432.CCR-15-0612

27. Nelson R. New Leukemia Drug Tops the Charts With a $178,000 Price Tag. In: Medscape [Internet]. 18 Dec 2014 [cited 14 Feb 2019]. Available: http://www.medscape.com/viewarticle/836879

28. Prasad V. Tisagenlecleucel — the first approved CAR-T-cell therapy: implications for payers and policy makers. Nature Reviews Clinical Oncology. 2018;15: 11–12. doi:10.1038/nrclinonc.2017.156

29. Cavallo J. Weighing the Cost and Value of CAR T-Cell Therapy. In: The ASCO Post [Internet]. 25 May 2018 [cited 14 Feb 2019]. Available: http://www.ascopost.com/issues/may-25-2018/weighing-the-cost-and-value-of-car-t-cell-therapy/

30. Koong AC, Mehta VK, Le QT, Fisher GA, Terris DJ, Brown JM, et al. Pancreatic tumors show high levels of hypoxia. International Journal of Radiation Oncology. 2000;48: 919–922. doi:10.1016/S0360-3016(00)00803-8

31. Hiraoka N, Ino Y, Sekine S, Tsuda H, Shimada K, Kosuge T, et al. Tumour necrosis is a postoperative prognostic marker for pancreatic cancer patients with a high interobserver reproducibility in histological evaluation. Br J Cancer. 2010;103: 1057–1065. doi:10.1038/sj.bjc.6605854

32. LaRocca CJ, Warner SG. A New Role for Vitamin D: The Enhancement of Oncolytic Viral Therapy in Pancreatic Cancer. Biomedicines. 2018;6. doi:10.3390/biomedicines6040104

33. Pancreatic cancer statistics - Canadian Cancer Society [Internet]. [cited 26 Feb 2019]. Available: http://www.cancer.ca/en/cancer-information/cancer-type/pancreatic/statistics/?region=on

34. Herreros-Villanueva M, Gironella M, Castells A, Bujanda L. Molecular markers in pancreatic cancer diagnosis. Clinica Chimica Acta. 2013;418: 22–29. doi:10.1016/j.cca.2012.12.025

35. Brand RE, Nolen BM, Zeh HJ, Allen PJ, Eloubeidi MA, Goldberg M, et al. Serum Biomarker Panels for the Detection of Pancreatic Cancer. Clin Cancer Res. 2011;17: 805–816. doi:10.1158/1078-0432.CCR-10-0248

36. Nakamura S, Sadakari Y, Ohtsuka T, Okayama T, Nakashima Y, Gotoh Y, et al. Pancreatic Juice Exosomal MicroRNAs as Biomarkers for Detection of Pancreatic Ductal Adenocarcinoma. Ann Surg Oncol. 2019; doi:10.1245/s10434-019-07269-z

37. Ettrich TJ, Berger AW, Schwerdel D, Reinacher-Schick AC, Uhl W, Alguel H, et al. A blood-based assay for diagnosis of early-stage pancreatic cancer. JCO. 2019;37: 234–234. doi:10.1200/JCO.2019.37.4_suppl.234

38. Faller BA, Burtness B. Treatment of pancreatic cancer with epidermal growth factor receptor-targeted therapy. Biologics. 2009;3: 419–428.

39. Heckler M, Dougan SK. Unmasking Pancreatic Cancer: Epitope Spreading After Single Antigen Chimeric Antigen Receptor T-Cell Therapy in a Human Phase I Trial. Gastroenterology. 2018;155: 11–14. doi:10.1053/j.gastro.2018.06.023