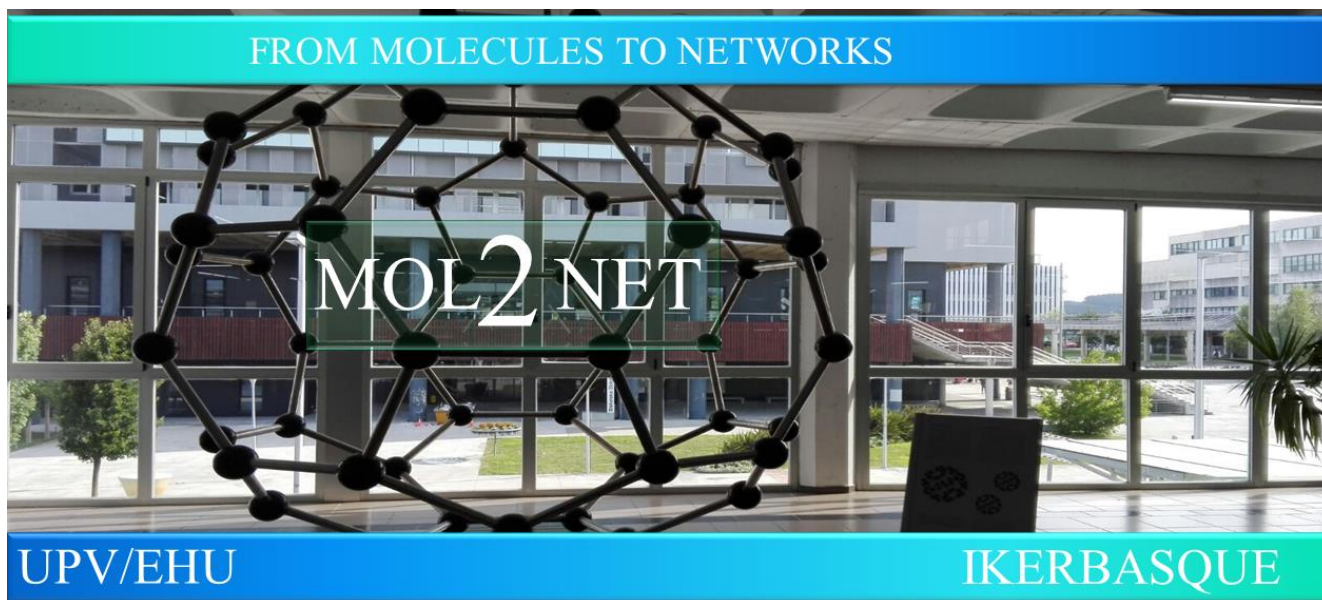




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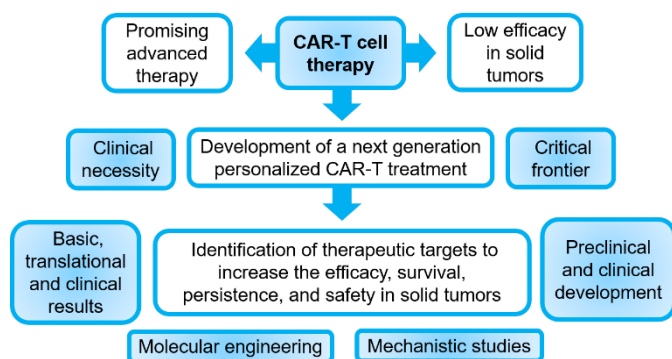
Clinical and preclinical development of novel and emerging CAR-T cell therapies for the treatment of solid tumors

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Graphical Abstract**Abstract.**

Adoptive cell therapy with genetically modified T lymphocytes that express chimeric antigen receptors is one of the most promising advanced therapies for cancer treatment. However, CAR-T cells efficacy in the solid tumor landscape is still very unsatisfactory. The development of a next generation personalized CAR-T treatment and the identification of therapeutic targets to increase the efficacy, survival, persistence, and safety in solid tumors remains as a clinical necessity and a critical frontier in cancer immunotherapy. Here, we summarize the basic, translational and clinical results of CAR-T cell immunotherapy for solid neoplasms, from its preclinical to its clinical development.

References

<https://clinicaltrials.gov/>

Arasanz H, Zuazo M, Bocanegra A, Chocarro L, Blanco E, Martínez M, Morilla I, Fernández G, Teijeira L, Morente P, Echaide M, Castro N, Fernández L, Garnica M, Ramos P, Escors D, Kochan G, Vera R. Hyperprogressive Disease: Main Features and Key Controversies. *Int J Mol Sci.* 2021 Apr 3;22(7):3736. doi: 10.3390/ijms22073736. PMID: 33916696; PMCID: PMC8038385.

Arasanz H, Zuazo M, Bocanegra A, Gato M, Martínez-Aguillo M, Morilla I, Fernández G, Hernández B, López P, Alberdi N, Hernández C, Chocarro L, Teijeira L, Vera R, Kochan G, Escors D. Early Detection of Hyperprogressive Disease in Non-Small Cell Lung Cancer by Monitoring of Systemic T Cell Dynamics. *Cancers (Basel).* 2020 Feb 4;12(2):344. doi: 10.3390/cancers12020344. PMID: 32033

Bocanegra A, Blanco E, Fernandez-Hinojal G, Arasanz H, Chocarro L, Zuazo M, Morente P, Vera R, Escors D, Kochan G. PD-L1 in Systemic Immunity: Unraveling Its Contribution to PD-1/PD-L1 Blockade Immunotherapy. *Int J Mol Sci.* 2020 Aug 18;21(16):5918. doi: 10.3390/ijms21165918. PMID: 32824655; PMCID: PMC7460585.

Brentjens RJ, Latouche JB, Santos E, Marti F, Gong MC, Lyddane C, King PD, Larson S, Weiss M, Rivière I, Sadelain M. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. *Nat Med.* 2003 Mar;9(3):279-86. doi: 10.1038/nm827. Epub 2003 Feb 10. PMID: 12579196.

Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther.* 2015;15(8):1145-54. doi: 10.1517/14712598.2015.1046430. Epub 2015 May 18. PMID: 25985798.

Chmielewski M, Hombach AA, Abken H. Of CARs and TRUCKs: chimeric antigen receptor (CAR) T cells engineered with an inducible cytokine to modulate the tumor stroma. *Immunol Rev.* 2014 Jan;257(1):83-90. doi: 10.1111/imr.12125. PMID: 24329791.

Chmielewski M, Kopecky C, Hombach AA, Abken H. IL-12 release by engineered T cells expressing chimeric antigen receptors can effectively Muster an antigen-independent macrophage response on tumor cells that have shut down tumor antigen expression. *Cancer Res.* 2011 Sep 1;71(17):5697-706. doi: 10.1158/0008-5472.CAN-11-0103. Epub 2011 Jul 8. PMID: 21742772.

Chocarro de Erauso L, Zuazo M, Arasanz H, Bocanegra A, Hernandez C, Fernandez G, Garcia-Granda MJ, Blanco E, Vera R, Kochan G, Escors D. Resistance to PD-L1/PD-1 Blockade Immunotherapy. A Tumor-Intrinsic or Tumor-Extrinsic Phenomenon? *Front Pharmacol.* 2020 Apr 7;11:441. doi: 10.3389/fphar.2020.00441. PMID: 32317979; PMCID: PMC7154133.

Chocarro L, Blanco E, Zuazo M, Arasanz H, Bocanegra A, Fernández-Rubio L, Morente P, Fernández-Hinojal G, Echaide M, Garnica M, Ramos P, Vera R, Kochan G, Escors D. Understanding LAG-3 Signaling. *Int J Mol Sci.* 2021 May 17;22(10):5282. doi: 10.3390/ijms22105282. PMID: 34067904; PMCID: PMC8156499.

De Munter S, Ingels J, Goetgeluk G, Bonte S, Pille M, Weening K, Kerre T, Abken H, Vandekerckhove B. Nanobody Based Dual Specific CARs. *Int J Mol Sci.* 2018 Jan 30;19(2):403. doi: 10.3390/ijms19020403. PMID: 29385713; PMCID: PMC5855625.

Duan D, Wang K, Wei C, Feng D, Liu Y, He Q, Xu X, Wang C, Zhao S, Lv L, Long J, Lin D, Zhao A, Fang B, Jiang J, Tang S, Gao J. The BCMA-Targeted Fourth-Generation CAR-T Cells Secreting IL-7 and CCL19 for Therapy of Refractory/Recurrent Multiple Myeloma. *Front Immunol.* 2021 Mar 5;12:609421. doi: 10.3389/fimmu.2021.609421. PMID: 33767695; PMCID: PMC7985831.

Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci U S A.* 1993 Jan 15;90(2):720-4. doi: 10.1073/pnas.90.2.720. PMID: 8421711; PMCID: PMC45737.

Feng KC, Guo YL, Liu Y, Dai HR, Wang Y, Lv HY, Huang JH, Yang QM, Han WD. Cocktail treatment with EGFR-specific and CD133-specific chimeric antigen receptor-modified T cells in a patient with advanced cholangiocarcinoma. *J Hematol Oncol.* 2017 Jan 5;10(1):4. doi: 10.1186/s13045-016-0378-7. PMID: 28057014; PMCID: PMC5217546.

Gargett T, Brown MP. The inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities of chimeric antigen receptor T cells. *Front Pharmacol.* 2014 Oct 28;5:235. doi: 10.3389/fphar.2014.00235. PMID: 25389405; PMCID: PMC4211380.

Grada Z, Hegde M, Byrd T, Shaffer DR, Ghazi A, Brawley VS, Corder A, Schönfeld K, Koch J, Dotti G, Heslop HE, Gottschalk S, Wels WS, Baker ML, Ahmed N. TanCAR: A Novel Bispecific Chimeric Antigen Receptor for Cancer Immunotherapy. *Mol Ther Nucleic Acids.* 2013 Jul 9;2(7):e105. doi: 10.1038/mtna.2013.32. PMID: 23839099; PMCID: PMC3731887.

Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proc Natl Acad Sci U S A.* 1989 Dec;86(24):10024-8. doi: 10.1073/pnas.86.24.10024. PMID: 2513569; PMCID: PMC298636.

Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. *EMBO Mol Med.* 2017 Sep;9(9):1183-1197. doi: 10.15252/emmm.201607485. PMID: 28765140; PMCID: PMC5582407.

Hartmann J, Schüßler-Lenz M, Bondanza A, Buchholz CJ. Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. *EMBO Mol Med.* 2017 Sep;9(9):1183-1197. doi: 10.15252/emmm.201607485. PMID: 28765140; PMCID: PMC5582407.

Hegde M, Corder A, Chow KK, Mukherjee M, Ashoori A, Kew Y, Zhang YJ, Baskin DS, Merchant FA, Brawley VS, Byrd TT, Krebs S, Wu MF, Liu H, Heslop HE, Gottschalk S, Yvon E, Ahmed N. Combinational targeting offsets antigen escape and enhances effector functions of adoptively transferred T cells in glioblastoma. *Mol Ther*. 2013 Nov;21(11):2087-101. doi: 10.1038/mt.2013.185. Epub 2013 Aug 13. Erratum in: *Mol Ther*. 2014 May;22(5):1063. Gottschalk, Stephen [corrected to Gottschalk, Stephen]. PMID: 23939024; PMCID: PMC3831041.

Hegde M, Mukherjee M, Grada Z, Pignata A, Landi D, Navai SA, Wakefield A, Fousek K, Bielamowicz K, Chow KK, Brawley VS, Byrd TT, Krebs S, Gottschalk S, Wels WS, Baker ML, Dotti G, Mamonkin M, Brenner MK, Orange JS, Ahmed N. Tandem CAR T cells targeting HER2 and IL13R α 2 mitigate tumor antigen escape. *J Clin Invest*. 2016 Aug 1;126(8):3036-52. doi: 10.1172/JCI83416. Epub 2016 Jul 18. PMID: 27427982; PMCID: PMC4966331.

Hernandez C, Arasanz H, Chocarro L, Bocanegra A, Zuazo M, Fernandez-Hinojal G, Blanco E, Vera R, Escors D, Kochan G. Systemic Blood Immune Cell Populations as Biomarkers for the Outcome of Immune Checkpoint Inhibitor Therapies. *Int J Mol Sci*. 2020 Mar 31;21(7):2411. doi: 10.3390/ijms21072411. PMID: 32244396; PMCID: PMC7177687.

Huang R, Li X, He Y, Zhu W, Gao L, Liu Y, Gao L, Wen Q, Zhong JF, Zhang C, Zhang X. Recent advances in CAR-T cell engineering. *J Hematol Oncol*. 2020 Jul 2;13(1):86. doi: 10.1186/s13045-020-00910-5. PMID: 32616000; PMCID: PMC7333410.

Jia H, Wang Z, Wang Y, Liu Y, Dai H, Tong C, Guo Y, Guo B, Ti D, Han X, Yang Q, Wu Z, Han W. Haploidentical CD19/CD22 bispecific CAR-T cells induced MRD-negative remission in a patient with relapsed and refractory adult B-ALL after haploidentical hematopoietic stem cell transplantation. *J Hematol Oncol*. 2019 Jun 10;12(1):57. doi: 10.1186/s13045-019-0741-6. PMID: 31182121; PMCID: PMC6558895.

Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, White DE, Wunderlich JR, Canevari S, Rogers-Freezer L, Chen CC, Yang JC, Rosenberg SA, Hwu P. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. *Clin Cancer Res*. 2006 Oct 15;12(20 Pt 1):6106-15. doi: 10.1158/1078-0432.CCR-06-1183. PMID: 17062687; PMCID: PMC2154351.

Lamers CH, van Elzakker P, Langeveld SC, Sleijfer S, Gratama JW. Process validation and clinical evaluation of a protocol to generate gene-modified T lymphocytes for immunogene therapy for metastatic renal cell carcinoma: GMP-controlled transduction and expansion of patient's T lymphocytes using a carboxy anhydrase IX-specific scFv transgene. *Cytotherapy*. 2006;8(6):542-53. doi: 10.1080/14653240601056396. PMID: 17148030.

Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer*. 2021 Mar;21(3):145-161. doi: 10.1038/s41568-020-00323-z. Epub 2021 Jan 22. PMID: 33483715; PMCID: PMC8353572.

Li D, Li X, Zhou WL, Huang Y, Liang X, Jiang L, Yang X, Sun J, Li Z, Han WD, Wang W. Genetically engineered T cells for cancer immunotherapy. *Signal Transduct Target Ther*. 2019 Sep 20;4:35. doi: 10.1038/s41392-019-0070-9. PMID: 31637014; PMCID: PMC6799837.

Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, Royal RE, Topalian SL, Kammula US, Restifo NP, Zheng Z, Nahvi A, de Vries CR, Rogers-Freezer LJ, Mavroukakis SA, Rosenberg SA. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science*. 2006 Oct 6;314(5796):126-9. doi: 10.1126/science.1129003. Epub 2006 Aug 31. PMID: 16946036; PMCID: PMC2267026.

Nair R, Westin J. CAR T-Cells. *Adv Exp Med Biol*. 2020;1244:215-233. doi: 10.1007/978-3-030-41008-7_10. PMID: 32301017.

Park JR, Digiusto DL, Slovak M, Wright C, Naranjo A, Wagner J, Meechooet HB, Bautista C, Chang WC, Ostberg JR, Jensen MC. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. *Mol Ther*. 2007 Apr;15(4):825-33. doi: 10.1038/sj.mt.6300104. Epub 2007 Feb 13. PMID: 17299405.

Ruella M, Barrett DM, Kenderian SS, Shestova O, Hofmann TJ, Perazzelli J, Klichinsky M, Aikawa V, Nazimuddin F, Kozlowski M, Scholler J, Lacey SF, Melenhorst JJ, Morrissette JJ, Christian DA, Hunter CA, Kalos M, Porter DL, June CH, Grupp SA, Gill S. Dual CD19 and CD123 targeting prevents antigen-loss relapses after CD19-directed immunotherapies. *J Clin Invest*. 2016 Oct 3;126(10):3814-3826. doi: 10.1172/JCI87366. Epub 2016 Aug 29. PMID: 27571406; PMCID: PMC5096828.

Sadelain M, Rivière I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. *Nat Rev Cancer*. 2003 Jan;3(1):35-45. doi: 10.1038/nrc971. PMID: 12509765.

Subklewe M, von Bergwelt-Baildon M, Humpe A. Chimeric Antigen Receptor T Cells: A Race to Revolutionize Cancer Therapy. *Transfus Med Hemother*. 2019 Feb;46(1):15-24. doi: 10.1159/000496870. Epub 2019 Feb 5. PMID: 31244578; PMCID: PMC6558337.

Tian Y, Li Y, Shao Y, Zhang Y. Gene modification strategies for next-generation CAR T cells against solid cancers. *J Hematol Oncol*. 2020 May 18;13(1):54. doi: 10.1186/s13045-020-00890-6. PMID: 32423475; PMCID: PMC7236186.

Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer*. 2016 May;16(5):275-87. doi: 10.1038/nrc.2016.36. Epub 2016 Apr 15. PMID: 27079802; PMCID: PMC5381938.

van der Stegen SJ, Hamieh M, Sadelain M. The pharmacology of second-generation chimeric antigen receptors. *Nat Rev Drug Discov*. 2015 Jul;14(7):499-509. doi: 10.1038/nrd4597. PMID: 26129802; PMCID: PMC6410718.

Wang Z, Cao YJ. Adoptive Cell Therapy Targeting Neoantigens: A Frontier for Cancer Research. *Front Immunol*. 2020 Mar 5;11:176. doi: 10.3389/fimmu.2020.00176. PMID: 32194541; PMCID: PMC7066210.

Yeku OO, Brentjens RJ. Armored CAR T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy. *Biochem Soc Trans*. 2016 Apr 15;44(2):412-8. doi: 10.1042/BST20150291. PMID: 27068948; PMCID: PMC5529098.

Zah E, Lin MY, Silva-Benedict A, Jensen MC, Chen YY. T Cells Expressing CD19/CD20 Bispecific Chimeric Antigen Receptors Prevent Antigen Escape by Malignant B Cells. *Cancer Immunol Res*. 2016 Jun;4(6):498-508. doi: 10.1158/2326-6066.CIR-15-0231. Epub 2016 Apr 8. PMID: 27059623; PMCID: PMC4933590.

Zeltsman M, Dozier J, McGee E, Ngai D, Adusumilli PS. CAR T-cell therapy for lung cancer and malignant pleural mesothelioma. *Transl Res*. 2017 Sep;187:1-10. doi: 10.1016/j.trsl.2017.04.004. Epub 2017 Apr 26. PMID: 28502785; PMCID: PMC5581988.

Zuazo M, Arasanz H, Bocanegra A, Fernandez G, Chocarro L, Vera R, Kochan G, Escors D. Systemic CD4 Immunity as a Key Contributor to PD-L1/PD-1 Blockade Immunotherapy Efficacy. *Front Immunol*. 2020 Nov 30;11:586907. doi: 10.3389/fimmu.2020.586907. PMID: 33329566; PMCID: PMC7734243.

Zuazo M, Arasanz H, Fernández-Hinojal G, García-Granda MJ, Gato M, Bocanegra A, Martínez M, Hernández B, Teijeira L, Morilla I, Lecumberri MJ, Fernández de Lascoiti A, Vera R, Kochan G, Escors D. Functional systemic CD4 immunity is required for clinical responses to PD-L1/PD-1 blockade therapy. *EMBO Mol Med*. 2019 Jul;11(7):e10293. doi: 10.15252/emmm.201910293. Epub 2019 Jun 6. PMID: 31273938; PMCID: PMC6609910