Engineering a Bispecific Claudin 18.2/TGF-β CAR to Target Claudin 18.2 Tumors and Overcome the Tumor Microenvironment

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Abstract

Gastric cancer (GC) is among the top three deadliest cancers and has the fifth-highest global cancer incidence (1). Claudin 18.2 (CLDN 18.2) is an isoform of the tight-junction protein Claudin 18 and is emerging as a promising target for CAR T cell therapies against GC. CLDN18.2 shows limited expression in normal tissues but is upregulated during malignant transformation as seen in GC, making CLDN18.2 an attractive tumor-associated antigen. (2). Recently, a clinical trial (NCT03874897) used CLDN18.2 CAR T cells to treat patients with GC and pancreatic cancer. In the GC population, the overall response rate and disease-control rate were 57.1% and 75.0%, respectively, with a median progression-free survival of 4.2 months among 28 treated patients (3). Repeat infusions of the CAR T product was provided to 39% of patients, which speaks to the potential need to enhance T cell persistence and function in solid tumors.

Studies have shown that GC patients have elevated levels of transforming growth factor-beta (TGF-β) in their gastric mucosa (4). This highly pleiotropic cytokine has profound immunosuppressive effects that limit T-cell persistence while also promoting tumor survival, immune evasion, and metastasis. Moreover, TGF-β induces the differentiation of CD4+ T cells into regulatory T cells (Tregs) that in turn attenuate immune activation and production of proinflammatory mediators (5). We hypothesized that, by engineering T cells to convert the TGF-β anti-inflammatory signal into a pro-inflammatory signal, we could significantly ameliorate TGF-β's inhibitory effects and enhance T-cell function, proliferation, and infiltration of CAR T cells in gastric tumors.

We designed a bispecific, OR-gate CAR to target both CLDN18.2 and TGF-β. The CLDN18.2/TGF-β bispecific CAR T cells were co-cultured with either CLDN18.2-positive gastric-cancer cell lines or with different isoforms of TGF-β. When co-cultured with CLDN18.2 cell lines, both the single-input (CLDN18.2 only) and bispecific CAR T cells showed high cytotoxicity with similar levels of T-cell activation, as evaluated by IFN-γ secretion. In addition, bispecific CAR T cells demonstrated reduced exhaustion markers and fewer Tregs compared to the CAR T cells that solely target CLDN18.2.

In summary, we show that the CLDN18.2/TGF-β bispecific CAR converts the immunosuppressive TGF-β signal into an activation signal, thereby enhancing T-cell fitness, reducing T-cell conversion into Tregs, and decreasing phenotypic exhaustion.

Methodology

Healthy-donor leukapheresis products were processed to enrich CD62L+ T cells. These enriched cells were transduced using a lentiviral vector system, which encoded a bispecific CAR targeting CLDN18.2 and TGF-β (IMPT-601). Control CAR T cells were simultaneously produced to express either a CLDN18.2 single-input CAR[1] or a benchmark CAR[2]. Following the manufacturing process, we harvested the CAR+ T cells and evaluated specificity, cytotoxicity, and phenotypic fitness.

CAR T cells were co-cultured with either recombinant TGF-β protein or an engineered human gastric cancer cell line (AGS) that expresses either of the CLDN18 isoforms (CLDN18.1 or CLDN18.2). Effectiveness of the CAR construct was assessed by measuring T-cell proliferation and IFN-γ secretion.

To assess T-cell fitness, T cells were co-cultured with a human GC cell line (GSU) that endogenously expresses CLDN18.2 and secretes active TGF-β. Two in vitro systems were utilized: repeated antigen challenges (RACs) and a three-dimensional (3D) spheroid model. In the RAC system, co-cultures were established at a 1:1 ratio of CAR+ T cell : GSU. After 2-3 days of incubation, the T-cell–rich supernatants were harvested and transferred to a new GSU challenge. At each RAC, a 12-point effector-to-target (E:T) titration was concurrently established to monitor cytotoxic sensitivity.

Spheroids were generated by seeding GSU cells in a 96-well, ultra-low attachment (ULA), round-bottom plate containing basement membrane extract (BME) for 1-2 days. After spheroid formation, T cells were titrated and seeded, with the highest E:T ratio at 10:1.

T cells were evaluated for Treg differentiation and T-cell exhaustion at the endpoint of RAC experiments and spheroid co-cultures.

[1]CLDN18.2 single-input CAR: CLDN18.2 targeting CAR, containing the parental CLDN18.2-binding scFv of IMPT-601.

[2]Benchmark CAR: CAR containing a clinically evaluated CLDN18.2-binding domain.

Abbreviations: BME = basement membrane extract, GFP = green fluorescent protein, Hz = humanized, ULA = ultra low attachment, UTD = untransduced v/v = volume/volume

Schematic of IMPT-601

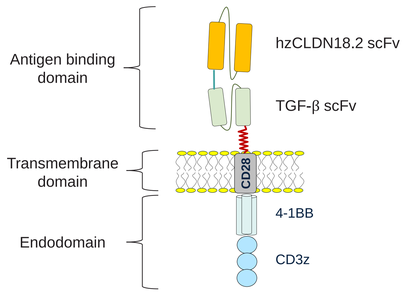

Figure 1. IMPT-601 is a second-generation, bispecific, OR-gate CAR. The extracellular domain is comprised of two linked single-chain variable fragments (scFvs) targeting CLDN18.2 and soluble TGF-β. IMPT-601 has a CD28 transmembrane domain, a 4-1BB co-stimulatory domain, and a CD3ζ activation domain.

IMPT-601 delivers target-specific T-cell activation in the presence of CLDN18.2 or TGF-β

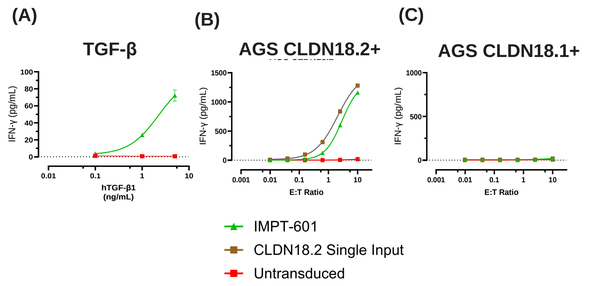

Figure 2. IMPT-601 CAR T cells demonstrate specific activation when cultured with either TGF-β or CLDN18.2. **(A)** T cells were spiked with varying concentrations of huTGF-β1. IMPT-601 CAR T cells secrete IFN-γ in a dose-dependent manner. **(B)** IFN-γ secretion of IMPT-601 CAR T cells co-cultured with AGS CLDN18.2+ gastric cell line. IMPT-601 and single input CLDN18.2 CAR T cells show similar levels of IFN-γ production. **(C)** Low/no detectable levels of IFN-γ levels were produced when co-cultured with the AGS CLDN18.1+ gastric cell line.

IMPT-601 completely clears tumor spheroids and exhibits marked reduction in exhausted and immunosuppressive T-cell phenotypes

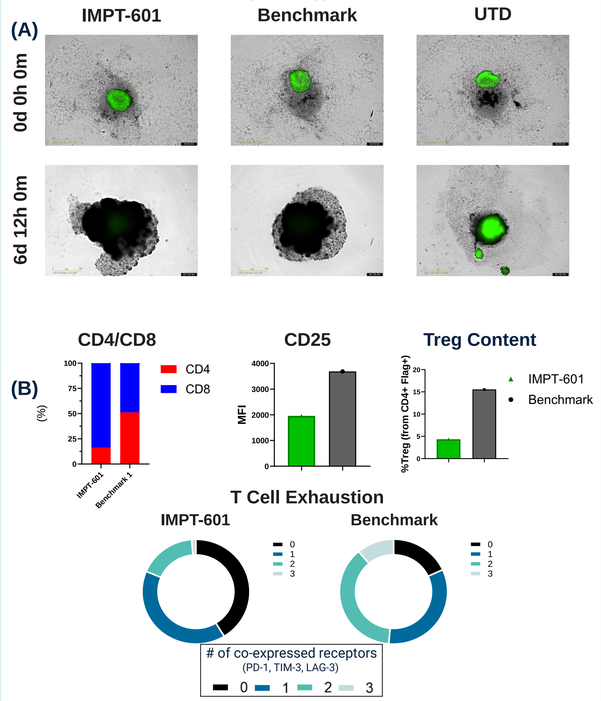

Figure 5. Spheroids were generated by seeding GSU gastric cell line, expressing GFP, in a ULA 96-well plate at 3,000 cells/well with 2.5%(v/v) BME for 1-2 days. T cells were added to the GSU spheroids. **(A)** GSU cells (GFP) are cleared by CAR T cells after 6 days. Images were obtained by the Incucyte SX5. **(B)** IMPT-601 CAR T cells have lower %CD4, reduced CD25 MFI, and lower Treg content and exhaustion marker expression at experimental endpoint.

IMPT-601 demonstrates persistent cytotoxicity across repeated antigen challenges (RACs)

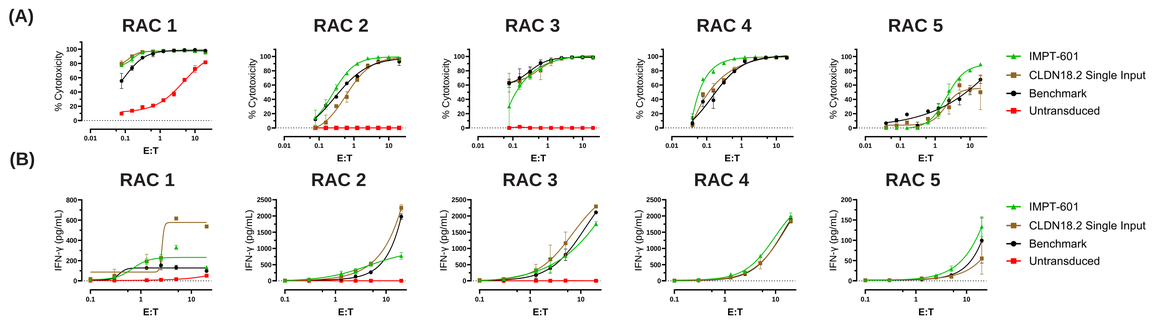

Figure 3. IMPT-601 is highly efficacious across five GSU RACs. **(A)** To establish T-cell killing potency, T cells were co-cultured with GSU gastric cell line across a 12-point E:T titration. At RAC 5, IMPT-601 CAR T cells demonstrate enhanced cytolytic characteristics compared to the other constructs, reaching nearly complete tumor clearance at higher E:T ratios. Single input control CARs, show a slight decrease in killing efficacy, reaching approximately 60-80% tumor clearance at the highest E:T ratio. **(B)** CAR+ T cells secrete IFN-γ at detectable levels when co-cultured with GSU gastric cell line. IFN-γ levels vary between challenges but are similar across most of the CAR T cell constructs. UTD T cells were discontinued at RAC 3 due to lack of T cell expansion.

IMPT-601 reduces regulatory T cell differentiation and phenotypic exhaustion

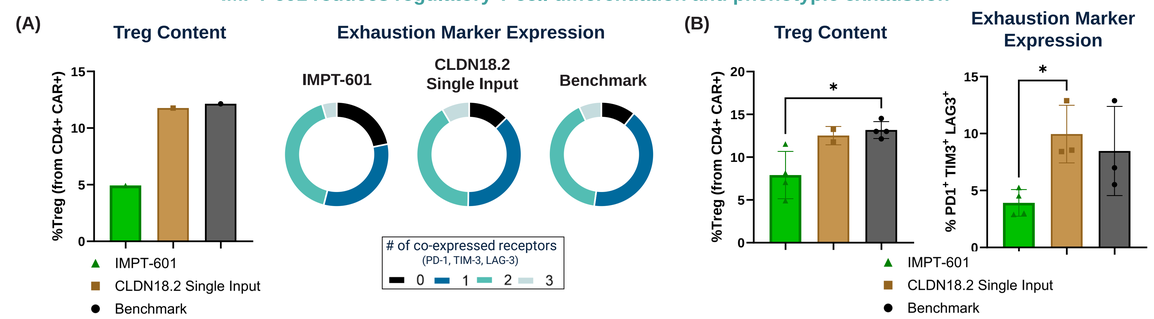

Figure 4. Regulatory and exhausted T-cell phenotypes were evaluated at the endpoint of all RACs using flow cytometry. **(A)** Representative Treg (left) and exhausted (right) T-cell quantification. **(B)** Treg content (left) and exhaustion marker expression (right) across three donors at the end of RAC5. In both (A) and (B), the IMPT-601 CAR T cells exhibit significantly reduced Treg content when compared to CLDN18.2 benchmark. Likewise, the co-expression of exhaustion markers in IMPT-601 CAR T cells is lower when compared to the CLDN18.2 single-input control. Means of triplicates ±1 standard deviation are shown. Statistical significance was calculated using unpaired one-way ANOVA. Tregs are defined as CD4+, CD25+, CD127- and FOXP3+

Conclusions

IMPT-601, a bispecific OR-gate CAR targeting both CLDN18.2 and TGF-β protein, demonstrated robust activation, proliferation, and IFN-γ secretion in response to either TGF-β protein or CLDN18.2 antigen. IMPT-601 reprograms an anti-inflammatory TGF-β signal into a pro-inflammatory signal, thus significantly enhancing anti-tumor immune responses in the immunosuppressive tumor microenvironment. Moreover, IMPT-601 maintained potent and sustained cytotoxicity against tumor cells over multiple rounds of antigen challenges. After 5 rounds of antigen challenges, IMPT-601 demonstrated reduced Treg differentiation and decreased exhaustion marker expression, suggesting that targeting of TGF-β protein, when combined with CLDN18.2 antigen targeting, could support persistent CAR T cell function despite extended antigen exposure. In summary, our data demonstrate the therapeutic potential of IMPT-601 against CLDN18.2-positive malignancies while overcoming the immunosuppressive tumor microenvironment.

References

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