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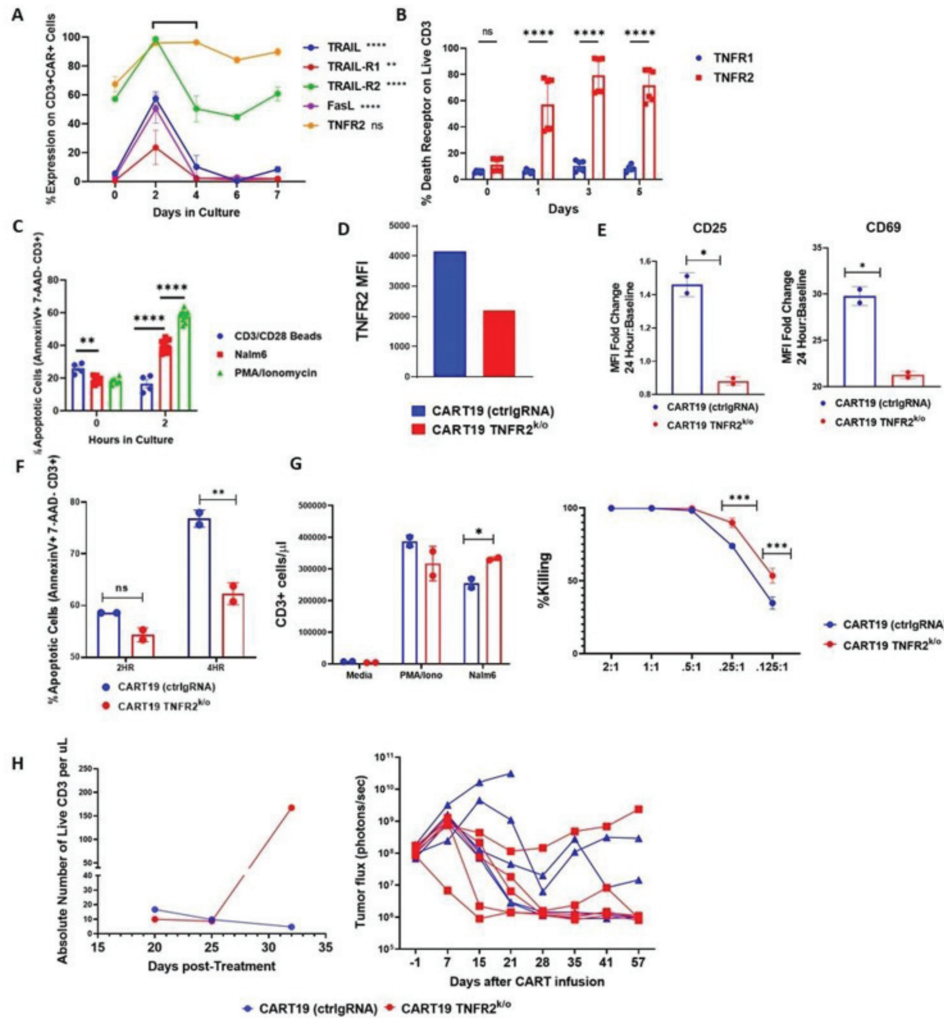

Fig 1. A). TNFR2 is persistently elevated, but not other death receptors, on CART19 cells using an extended *in vitro* culture model. CART19 cells from healthy donors were co-cultured with irradiated CD19+ Jeko-1 cells and repeatedly stimulated at days 0, 2, 4, and 6. Flow cytometric analysis was done in order to measure levels of death receptors during each time point (ns= not significant, ** $p < 0.01$**** $p < 0.0001$; two-way ANOVA). **B). TNFR2, but not TNFR1 is highly upregulated upon CART19 antigen-specific stimulation.** CART19 cells were co-cultured with irradiated Nalm6 (CD19+) cells. Flow cytometric analysis was performed in order to measure TNFR1 and TNFR2 levels at days 0,1,3 & 5 (ns= not significant, **** $p < 0.0001$; two-way ANOVA). **C). CART19 cells undergo more apoptosis upon antigen-specific stimulation in comparison to T-cell receptor (TCR) stimulation over time.** CART19 cells were co-cultured with either PMA/Ionomycin, CD3/CD28 beads (TCR stimulation) or $CD19^+$ cell line Nalm6. Flow cytometric staining for Annexin V and 7-AAD is performed at 0 and 2 hours (** $p < 0.01$, **** $p < 0.0001$; two-way ANOVA). **D). Disruption of TNFR2 on CART19 cells using CRISPR/Cas9.** Representative figure showing levels of TNFR2 from CART19 (ctrlgRNA) and CART19 $TNFR2^{k/o}$ cells at day 6 of production. **E). $TNFR2^{k/o}$ CART19 cells showed decreased levels of T cell activation markers in comparison to CART19(ctrlgRNA) cells.** $TNFR2^{k/o}$ or ctrlgRNA CART19 cells were co-culture with irradiated Nalm6 (CD19+) cell lines. Flow cytometric staining was performed at baseline (0HR) and after 24hrs in order to measure CD25 and CD69 (* $p<0.05$; t-test). **F). $TNFR2^{k/o}$ CART19 cells showed decreased levels of apoptosis in comparison to CART19(ctrlgRNA) cells.** CART19(ctrlgRNA) or $TNFR2^{k/o}$ CART19 cells were co-cultured with Nalm6 (CD19+) cell line. Flow cytometric staining for Annexin V and 7-AAD is performed at 2 and 4 hours (** $p < 0.01$; two-way ANOVA). **G). $TNFR2^{k/o}$ CART19 cells showed enhanced antigen-specific proliferation (A) and cytotoxicity (B) in comparison to CART19(ctrlgRNA) cells.** CART19(ctrlgRNA) or $TNFR2^{k/o}$ CART19 cells were co-cultured with irradiated Nalm6 (CD19+) cells, PMA/Ionomycin or Media alone. Flow cytometric staining was performed in order to measure absolute number of CD3+ cells (left panel). CART19(ctrlgRNA) or $TNFR2^{k/o}$ CART19 cells were co-cultured with Luciferase+ Nalm6 (CD19+) cells and cytotoxicity was measured, via bioluminescence analysis, after 48hrs (right panel; *$p<0.05$,** $p < 0.01$; two-way ANOVA. **H). $TNFR2^{k/o}$ CART19 cells showed improved CART cell expansion, enhanced anti-tumor activity and proliferation *in vivo* in comparison to CART19(ctrlgRNA) cells.** NSG mice were engrafted with JeKo-1 and then randomized to receive either CART19(ctrlgRNA) or $TNFR2^{k/o}$ CART19 cells. Tail bleeding was done once a week in order to assess CART cell expansion *in vivo* (left panel). Bioluminescence was measured once a week in order to assess burden of disease (right panel).

Fig. 1 (abstract 37).

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Immunotherapy

SAFETY AND EFFICACY OF SARS-COV-2-SPECIFIC T CELLS AS ADOPTIVE IMMUNOTHERAPY FOR HIGH-RISK COVID-19 PATIENTS: A PHASE I/II, RANDOMIZED CLINICAL TRIAL

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Keywords: SARS-CoV-2, Virus-Specific T Cells, Adoptive Immunotherapy.

Background & Aim: SARS-CoV-2 pandemic poses an urgent need for the development of effective therapies. We report the feasibility of creating a bank of immediately available off-the-shelf SARS-CoV-2-specific T cells (CoV-2-STs) from convalescents and preliminary results of a randomized phase I/II trial (EudraCT 2021-001022-22) using CoV-2- STs in high-risk COVID-19 patients.

Methods, Results & Conclusion: We prepared ~480 clinical doses of CoV-2-STs from 30 convalescent donors. Peripheral blood mononuclear cells were exposed to pepmixes spanning SARS-CoV-2 antigens (spike/membrane/NCAP) and expanded for 10 days in G-Rex to produce a median of $6x10^8$ T-cells/donor ($2-11x10^8$). The cell products were polyclonal, enriched in CD4+ (78±2%) cells expressing memory markers and high specificity against SARS-CoV-2 [2428±109 spot forming cells (SFC)/$2x10^5$] and its variants (WT 1873±481 /alpha 2182±582/beta 2177±624 /delta 1549±463 SFC/$2x10^5$). At least 1 HLA mediating CoV-2-ST specificity was identified in 29/30 products. Hospitalized COVID-19 patients within 6 days from the symptoms onset with pneumonia, lymphopenia (CD3+≤650/μl) and ≥1 elevated biomarker (D-dimers, ferritin, CRP, LDH) were enrolled and followed for 8 weeks. Patients were evaluated for recovery by the WHO 8-point Ordinal Scale (OS). Safety was demonstrated during phase I where 6 patients received dose escalated ($15x10^6$,$2x10^7/m^2$) CoV-2-STs sharing at least 1 HLA mediating specificity. In phase II, 90 randomized (2:1) high-risk patients were enrolled; 57 received the standard of care (SoC) plus partially HLA-matched CoV-2-STs and 30 received the SoC (control arm). Three withdrew consent and 1 from the CoV-2-ST arm was allocated to control arm as referred to ICU prior to receiving CoV-2-STs. On day 60 (d60) the add- on treatment resulted in 51% lower risk of mortality than SoC alone (24.6% [14/57] vs 50.0% [15/30]; risk ratio (RR): 0.49;p=0.016) with crude hazard ratio (HR) 2.42 (1.17, 5.05;p=0.018) in favor of CoV-2-STs. The benefit on survival was confirmed by multiple analysis after adjustment for confounding factors [HR:2.16(1.02, 4.60);p=0.04]. On d30, 67% had recovered (OS≤3) in the CoV-2-ST arm vs 37% in the control arm (RR, 1.82;p=0.02) with HR 0.48 (0.24, 0.94; p=0.03). CoV-2-ST-treated patients were more likely to recover by d30 even after adjustment for confounding factors [HR:0.46(0.23,0.92);p=0.03]. Overall, off-the-shelf immunotherapy with CoV-2-STs can serve as a safe and effective treatment in a real world environment for severe COVID-19.

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Immunotherapy

B7H3-CAR NK CELLS AND DNR CO-TRANSDUCED NK SHOWS MAINTAIN THEIR POTENCY AGAINST TGF-B MEDIATED IMMUNE SUPPRESSION

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Keywords: B7H3, Glioblastoma, TGF-B.

Background & Aim: Glioblastoma (GBM) is the most common form of primary brain tumor with a dismal overall survival. Allogeneic NK-cell-based immunotherapies are an attractive cell as they readily recognize non-self and stress ligands on malignant cells without damaging healthy cells. However, GBM employs multiple mechanisms to evade NK-cell targeting by downregulating NK-cell activating receptor ligands and secreting immune suppressive cytokines (e.g. TGF-β). Hence, we hypothesize that redirecting NK-cell specificity to the tumor-associated antigen B7H3 using a chimeric antigen receptor (CAR) approach and armoring them with a TGF-β dominant negative receptor (DNR) would overcome GBM-induced immune evasion to enhance NK-cell potency against this malignancy.

Methods, Results & Conclusion: NK cells co-transduced with the B7H3-CAR and DNR retroviral vectors were co- cultured with TGF-β and then evaluated for cytolytic activity against the GBM cell line U87 MG. B7-H3-CAR NK-cells generated from cord blood (CB) donor sources showed robust killing against GBM cells in vitro (89.73±2.44 % at 20:1 E:T ratio). Expectedly, they were impaired in the presence of TGF-β, as evidenced by significantly decreased expression of activation receptors (NKG2D expression downregulated by 68.14%±6.92%; p<0.05)) that markedly reduced cytolytic ability. Specifically, B7H3-CAR NK-cell cytotoxicity against U87 MG cells decreased from 89.73±2.44% to 61.75±3.42% at an effector to target ratio of 20:1 on exposure to TGF-β (p<0.05). After co-transducing CB- derived NK-cells to express the B7H3-CAR.CD28z and the TGF-β DNR (72.21% ± 23.05%), however, we observed that TGF-β DNR preserved the cytolytic function of B7H3 CAR NK cells in the presence of exogenous TGF-β (85.45%±3.05% cytotoxicity in the presence of TGF-B, vs 80.89% ±14.84% in the absence of TGF-β against U87 at E:T ratio 20:1, p>0.05). Besides abrogating decreased killing, DNR also mitigated against activating receptor downregulation. Only 3.83% ± 0.39% loss in CD16 receptor expression was observed in DNR-B7H3-CAR.CD28z NK-cells compared to 69.03%±4.63% for B7H3-CAR.CD28z NK-cells on exposure of exogenous TGF-β (p<0.05). In summary, this study shows that utilizing a DNR and CAR co-expression strategy on NK-cells may be a promising therapeutic advance for the treatment of GBM.

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Immunotherapy

A NOVEL GMP PROTOCOL USING THE HUMAN THYMUS AS A NEW SOURCE OF REGULATORY T CELLS (THYTREG) TO BE EMPLOYED AS AN AUTOLOGOUS CELLULAR THERAPY IN HEART TRANSPLANTED CHILDREN

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Keywords: Cell therapy, Treg, Clinical Trial.

Background & Aim: Immune allograft rejection continues to be the main obstacle to definite successful transplants. Due to their suppressive capacity, adoptive transfer of regulatory T cells (Treg) has acquired growing interest in achieving indefinite graft survival. Limited Treg recovery and reduced quality (in terms of survival, suppressive capacity and phenotype stability) remain the main obstacles in current protocols where Treg are obtained from adult peripheral blood. To overcome these limitations, we have developed a novel GMP-com-