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703.ADOPTIVE IMMUNOTHERAPY: MECHANISMS AND NEW APPROACHES | NOVEMBER 5, 2020

Using Allogeneic, Off-the-Shelf, Sars-Cov-2-Specific T Cells to Treat High Risk Patients with COVID-19

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Background. On 11 March, 2020 the World Health Organization declared COVID-19, caused by SARS-CoV-2, a global pandemic with almost 17,000,000 confirmed cases worldwide by the end of July, of which 4,500,000 were in the US. Approximately 20% of patients develop severe disease that can evolve into acute respiratory distress syndrome leading to respiratory or multiorgan failure, with an overall mortality of up to 4%. Older age, comorbidities such as hypertension and diabetes, and immune compromise have been identified as major risk factors associated with poor prognosis. For example, in immunocompromised HSCT patients mortality rates as high as 20% have been reported (www.cibmtr.org/COVID19). Furthermore, there is accumulating evidence regarding the protective role of T cells, with reduced counts and dysregulation seen more prominently in individuals with severe rather than mild COVID-19.

Our group has previously demonstrated the feasibility, safety and clinical efficacy of administering allogeneic ex vivo expanded multivirus-specific T cells (multi-VSTs) as a banked, off-the-shelf product for the treatment of EBV, CMV, BKV, HHV6 and AdV infections/disease in immunocompromised individuals. Given the lack of preventative or therapeutic agents and the emerging evidence of the pivotal protective role of SARS-CoV-2-specific CD4+ and CD8+ T cells, we sought to explore the feasibility of developing a

banked, SARS-CoV-2-specific VST product to treat those at highest risk of severe COVID-19 disease (i.e. HSCT recipients, elderly individuals, patients with comorbidities).

Methods. To first identify immunogenic and protective SARS-CoV-2 antigens we screened PBMCs from convalescent individuals with mild COVID-19 (not requiring hospitalization) for T cell activity against overlapping peptide libraries (pepmixes) spanning 18 structural and non-structural SARS-CoV-2 proteins of which 8 [structural proteins: Spike (S), Membrane (M) and Nucleoprotein (N); non-structural proteins (Nsps): 3, 4, 6, 12; and the accessory protein (AP) 7a] were identified as immunodominant and advanced for VST manufacturing. We subsequently utilized our optimized VST manufacturing process and culture in a G-Rex device in medium supplemented with activating cytokines to generate SARS-CoV-2-specific T cells with activity against this combination of immunodominant targets.

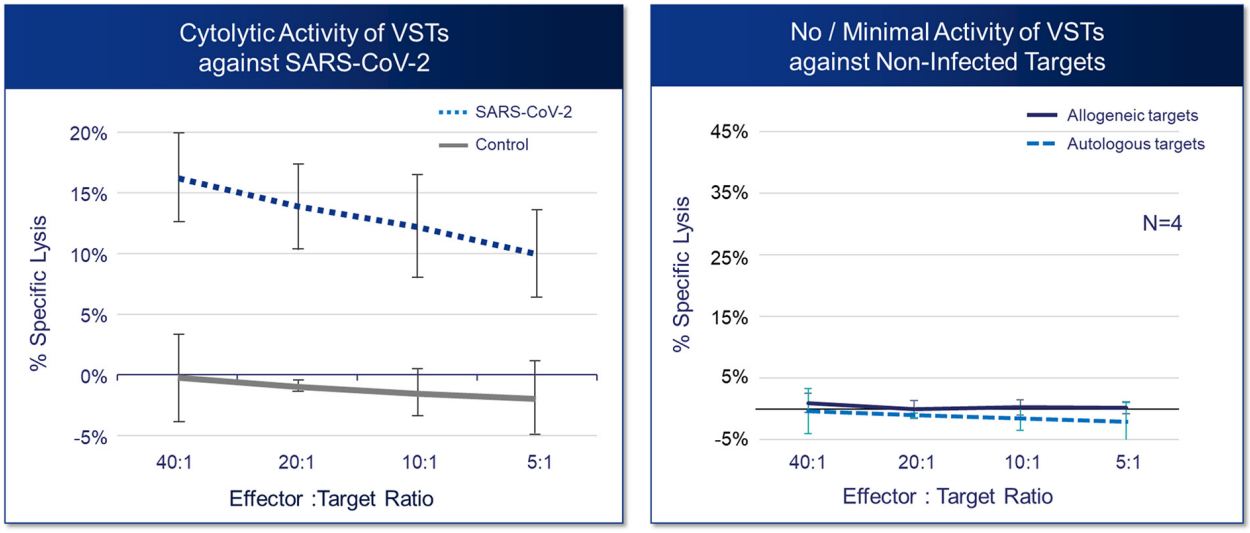
Results. We achieved a mean 29±7 fold expansion (mean±SEM; n=5) of cells that were comprised almost exclusively of CD3+ T cells (97.1±0.7%; mean±SEM), with a mixture of cytotoxic (CD8+; 10.2±1.2%) and helper (CD4+; 85.5±1.8%) T cells. These cells had a phenotype consistent with effector function and memory potential, as evidenced by upregulation of the activation markers CD25, CD69, and CD28 and expression of central (CD45RO+/CD62L+) and effector memory markers (CD45RO+/CD62L−), with minimal PD1 or Tim3 expression.

To confirm the anti-viral activity of our expanded cells we performed an IFNγ ELIspot using each of the individual stimulating antigens as an immunogen and all lines proved to be reactive against the target antigens [S: 2,118±479 SFC/2x10^5; M: 1,084±182; N: 1,124±335; Nsp3: 71±48.6; Nsp4: 68±30; Nsp6: 23±6.7; AP7a: 65±43; and Nsp12: 29±9]. As demonstrated by intracellular cytokine staining (ICS), the immune response was mediated by both CD4+ and CD8+ T cell subsets, and the majority of IFNg-producing cells also produced TNFa. Reactive cells exhibited a primarily Th1-polarized profile as measured by Granzyme B production, luminex array and single-cell protein analysis. In addition, the expanded cells were able to kill viral pepmix-loaded autologous PHA blasts with minimal/no activity against non-antigen-expressing autologous and allogeneic targets (Figure 1).

Conclusion. SARS-CoV-2 VSTs generated from convalescent individuals are Th1-polarized, polyfunctional and selectively able to kill viral antigen-expressing targets with no auto- or alloreactivity, indicative of both their selectivity and safety for clinical use. We are rapidly advancing this product to the clinic for administration in a randomized clinical trial (VSTs+SOC vs SOC) to prevent the development of severe disease in high risk hospitalized patients such as those post-transplant or therapy for hematologic malignancy.

Figure 1: SARS-CoV-2 Specific T cells Demonstrate Selective Cytolytic Activity against viral antigen-expressing targets.

Figure 1



Disclosures

Vasileiou: *AlloVir:* Consultancy. **Kuvalekar:** *AlloVir:* Consultancy. **Workineh:** *AlloVir:* Current Employment. **Watanabe:** *AlloVir:* Consultancy. **Heslop:** *Novartis:* Consultancy; *Gilead Biosciences:* Consultancy; *PACT Pharma:* Consultancy; *Kiadis:* Consultancy; *Tessa Therapeutics:* Consultancy, Research Funding; *AlloVir:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *Marker Therapeutics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees. **Hill:** *Incyte:* Membership on an entity's Board of Directors or advisory committees. **Leen:** *AlloVir:* Consultancy, Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees; *Marker Therapeutics:* Current equity holder in publicly-traded company, Membership on an entity's Board of Directors or advisory committees.

Author notes

* Asterisk with author names denotes non-ASH members.

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