691 MDK-271: A DUAL FUNCTION MOLECULE CONSISTING OF EMPIRICALLY-DESIGNED PEPTIDYL AGONISTS OF IL-2/15RβγC AND IL-7RαγC, UNRELATED TO IL-2, IL-15, OR IL-7, INCORPORATED INTO A BISPECIFIC FC FUSION PROTEIN

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Background Activation of IL-2/15Rβγc or IL-7R on immune cells using modified versions of IL-2 or IL-7 is under investigation as monotherapy, or in combination with checkpoint inhibitors, engineered T or NK cells, or neo-antigen vaccines. We previously described small synthetic peptides, unrelated to IL-2, IL-15, or IL-7, that selectively activate either IL-2/15Rβγc or IL-7R. IL-2/15Rβγc and IL-7R activation exhibit complementary effects on immune cells that when combined may offer benefits over each independent mechanism. We now report the creation of an Fc-fusion protein that incorporates both IL-2/15Rβγc and IL-7R agonist peptides, and characterize its properties in cell lines and human (PBMC) lymphocyte subpopulations.

Methods Peptide agonists of IL-2/15Rβγc (MDK1169) and IL-7R (MDK1319) were separately fused to each chain of obligate heterodimeric (asymmetric) Fc molecules. The Fc-fusion was purified by protein-A and size exclusion chromatography, and characterized by LC-MS. Receptor-mediated signaling, proliferation, and cell-surface receptor expression in cell lines, PBMCs, mixed and isolated lymphocyte subpopulations were determined by flow cytometry and ELISA to evaluate effects of IL-2, IL-2v, IL-7, MDK-202 or MDK-701 (Fc-fusions of MDK1169 and MDK1319, respectively), and combinations (mixtures) of these molecules, in comparison with the dual agonist MDK-271.

Results LC-MS analysis indicates MDK-271 is a heterodimeric molecule containing one copy each of MDK1169 (IL-2/15Rβγc-biased agonist) and MDK1319 (IL-7R agonist) fused to individual Fc-chains. Cell-based assay of MDK-271 demonstrates potent, fully efficacious phosphorylation of STAT5 in TF-1 cells expressing Rγc, and engineered to express either IL-2/15Rβ or IL-7Rα. PBMCs exposed ex vivo to MDK-271 exhibit additive, complementary, and synergistic effects among various lymphocyte subpopulations: CD4+Tn, Teff, Treg, Tmem; C8+Tn, Teff, Tmem; and NK cells, in this analysis. The mono-specific agonists MDK-202 and MDK-701 produce proliferative effects and signaling patterns in responsive cell lines and lymphocyte subsets similar to those induced by IL-2v (an IL-2/15Rβγc-biased mutant of IL-2) and IL-7, respectively. Combining both activities in MDK-271 induces response profiles that differ in some T-cell subsets from those of mono-specific agonists of the two receptors. Animal studies designed to understand the effects of these differences are underway.

Conclusions IL-2/15Rβγc and IL-7R are both currently undergoing extensive scrutiny as potential immuno-oncology therapeutic targets. The biology of these cytokines is both overlapping and complementary in stimulating and supporting T-cell populations; and some recent evidence suggests possible superiority of the combination. Based on in vitro properties, the Fc-peptide fusion reported here, exhibiting both IL-2/15Rβγc-biased agonist and Il-7Rαγc agonist activities, could be valuable in anti-tumor therapeutic applications.

Ethics Approval The use of human PBMC in this study was authorized under Minimal Risk Research Related Activities at Stanford Blood Center (SQL 79075)

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0691

692 AMV564, A CLINICALLY ACTIVE T CELL ENGAGER, INDUCES A TARGET-DEPENDENT ADAPTIVE IMMUNE RESPONSE

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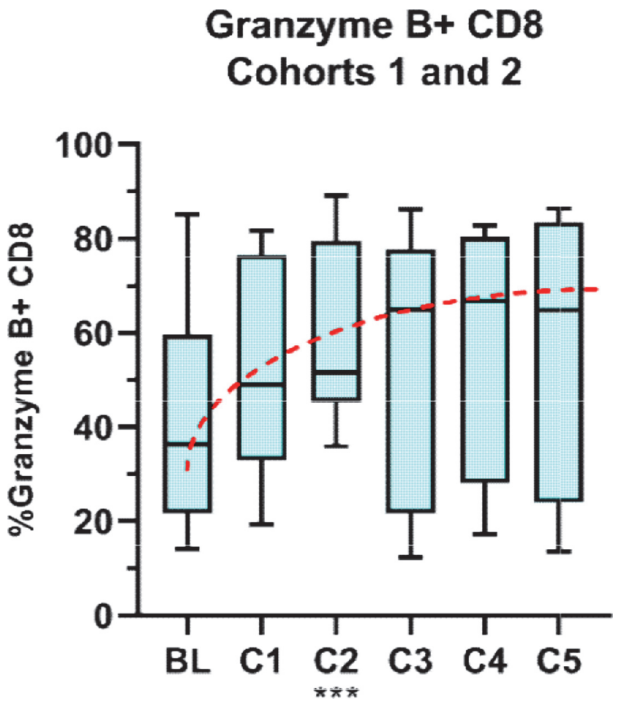
Background AMV564 is a potent bispecific T cell engager that binds CD3 and CD33. Due to its bivalent structure, AMV564 is selective for MDSCs via clustered CD33 expressed on the cell surface both in vitro and in patients. MDSCs are responsible for local and systemic suppression of the immune response to both circulating and solid cancers. Targeting MDSC suppression allows T cell priming to be restored in both the lymph nodes and tumor microenvironment, and expands previously activated tumor-specific T cells. Here we report clinical observations and results of our ex vivo assay development.

Methods Cell lines, primary human cells, and patient samples were analyzed using flow cytometry with appropriate marker panels including AMV564 directly labeled (phycoerythrin) or detected with labeled anti-AMV564 antibodies. T cell cytotoxicity assays were conducted using primary human T cells and leukemic blast or other target cells (3:1 ratio) for 48 or 72 hours. Patient peripheral blood was sequenced for TcRbeta CDR3 variable chain on the hsTCRBv4b.

Results AMV564 is currently under investigation in a Phase 1 clinical trial (NCT04128423). There have been no dose-limiting toxicities and clinical activity has been observed (RECIST complete response in an ovarian cancer patient) when dosed once daily as a subcutaneous injection. In patients, T cell redistribution is consistent with activation and depletion of both monocytic and granulocytic MDSCs. Immune profile changes consistent with CD8 and Th1 cell activation are observed (figure 1). Furthermore, TCR sequencing data



Abstract 692 Figure 1 Peripheral blood of a solid tumor patient shows robust activation of CD8 T cells over 5 cycles of AMV564 therapy. Significant increases in effector CD8 for patients treated with 15 or 50 mcg AMV564 as monotherapy (n = 8, *** p < 0.001)