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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

William Dower*, Steven Cwirla, Alice Bakker, Praechompoo Pongtornpipat, Blake Williams, Prarthana Joshi, Sandra Wang, Michael Needels, Ronald Barrett. *Medikine, Menlo Park, CA, USA*

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)

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

Sterling Eckard*, Aurelien Sarde, Li Mei, Curtis Ruegg, Patrick Chun, Victoria Smith. *Amphivena Therapeutics, South San Francisco, CA, USA*

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)

indicate that one cycle of treatment is sufficient to expand and generate de novo clones (figure 2). We developed a primary cell cytotoxicity assay and observe that cytotoxic potency is target dependent. Target cell killing and T cell activation/proliferation depend on CD33 clustering, and both CD4 and CD8 T cells can engage and kill target cells. This is illustrated in assays with KG-1 (M2, clustered) and KG-1a (M0, not clustered) cell lines, in which the KG-1 cells have an EC₅₀ 15–20 fold lower than the M0 cell line (figure 3). In addition, there is little to no detectable binding or killing of monocytes or neutrophils, which is consistent with the absence of neutropenia in patients enrolled in the trial to date.

Abstract 692 Figure 2 TcRb CDR3 sequencing of an ovarian cancer patient shows extensive clonal expansion upon treatment. Scatter plots represent clonal abundance in the periphery between Baseline (C1D1) and C1D12 using the differential Abundance tool (Adaptive Biotechnology)

Abstract 692 Figure 3 Cytotoxicity assay using fresh primary T cells demonstrates target selectivity on two cell lines with equivalent CD33 surface expression. T cell activation by AMV564 at clinically relevant doses is equivalent to CD3/CD28 stimulation

Conclusions AMV564 is a potent conditional T cell agonist which is clinically active. We demonstrate that the combination of T cell activation, increased T cell diversity, and target specificity allow AMV564 to deplete MDSCs and restore a native immune response to cancer.

Ethics Approval This study was approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) at each participating institution.

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693 APX601, A NOVEL TNFR2 ANTAGONIST ANTIBODY FOR CANCER IMMUNOTHERAPY

Erin Filbert*, Sushma Krishnan, Ryan Alvarado, George Huang, Francis Bahjat, Xiaodong Yang. Apexigen, Inc., San Carlos, CA, USA

Background A key barrier to effective immunotherapy for cancer is the immunosuppressive tumor microenvironment (TME) characterized by infiltrating regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSC). While depletion of immune-suppressive cells is a promising cancer immunotherapy strategy, current approaches are ineffective due to lack of specificity and safety concerns. Tumor Necrosis Factor Receptor 2 (TNFR2) is emerging as a novel, selective target to overcome immunosuppression in TME. TNFR2 expression is generally restricted to highly immunosuppressive cell populations in the TME and the TNFR2-TNF- α pathway plays an important role in the generation and survival of these cells. TNFR2 is also an oncogene upregulated on certain tumors and can enhance tumor cell survival. Thus, targeting TNFR2 is a promising therapeutic approach with multiple potential mechanisms of action.

Methods A diverse panel of antibodies to TNFR2 was created using APXiMAB™, Apexigen's proprietary rabbit monoclonal antibody technology. A robust assessment of over 100 antibody candidates for TNFR2 binding, TNF- α blockade and functional assays yielded APX601, a humanized IgG1 antibody, as the lead therapeutic candidate. The ability of APX601 to reverse immune suppression was assessed in Treg and MDSC suppression assays. In addition, the ability of APX601 to deplete TNFR2-expressing Treg and tumor cells was assessed both in vitro and in vivo using the mouse Colo205 xenograft model.

Results APX601 binds specifically to human TNFR2 with high affinity ($K_d = 47$ pM) and recognizes a unique epitope in the CRD1 domain of TNFR2. APX601 is a potent antagonist that blocks the TNFR2-TNF- α interaction in cell-based ligand binding assays ($IC_{50} = 0.149$ nM). APX601 is capable of reversing immune suppression via two mechanisms: 1) significant blockade of the immunosuppressive functions of both Tregs and MDSCs by inhibiting the binding of TNFR2 to its ligand TNF- α and 2) depletion of TNFR2-expressing Tregs, MDSC and tumor cells via antibody-dependent cell cytotoxicity (ADCC) ($EC_{50} = 1.14$ nM) and ADCP ($EC_{50} = 0.71$ nM) effector functions.

Conclusions APX601 is a potent TNFR2 antagonist antibody that reverses immune suppression by targeting TNFR2-expressing Treg and MDSC, and induces killing of tumor cells. Our data support the further development of APX601, a promising immunotherapeutic antibody with multiple potential mechanisms of action, for the treatment of a variety of solid tumors.

Ethics Approval Healthy human blood samples were obtained from Stanford Blood Center (Palo Alto, CA) from consenting donors under an approved protocol.

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694 NC410 IS A NOVEL IMMUNOMEDICINE FOR THE TREATMENT OF SOLID TUMORS

¹Linjie Tian, ²M Ines Pascoal Ramos, ²Emma de Ruiter, ¹Ana Paucarmayta, ²Eline Elshof, ²Stefan Willems, ¹Chang Song, ¹Zachary Cusumano, ¹Jason Bosiacki, ¹Linda Liu, ¹Solomon Langermann, ²Linde Meysaard, ¹Dallas Flies*. ¹NextCure, Inc., Beltsville, MD, USA; ²University Medical Center, Utrecht, Utrecht, Netherlands

Background Abnormalities in the extracellular matrix of tumor microenvironments support tumor progression, lead to