The Combination of a Mouse Ortholog of ALKS 4230, a Selective Agonist of the Intermediate-Affinity IL-2 Receptor, and the Angiogenesis Inhibitor Lucitanib Enhances Antitumor Activity

Jared E. Lopes,[1] Rachel L. Dusek,[2] Liliane Robillard,[2] Minh Nguyen,[2] Bruce Roth,[1] Bryan Vought,[1] Arthur Liberzon,[1] Raymond J. Winquist,[1] Heather C. Losey[1]

[1]Alkermes, Inc., Waltham, MA; [2]Clovis Oncology, Inc., Boulder, CO

Abstract 2202

INTRODUCTION

- ALKS 4230, a novel cytokine, is an investigational, engineered fusion protein designed to selectively bind to the intermediate-affinity IL-2R. ALKS 4230 is being evaluated as a monotherapy and in combination with pembrolizumab in patients with advanced solid tumors.[1]
- The selectivity of ALKS 4230 is achieved through stable fusion of circularly permuted IL-2 to the IL-2Rα chain, driving antitumor responses via selective activation of CD8⁺ T cells and NK cells, while avoiding activation of T_{regs}, which express the high-affinity IL-2R.[1]
- High levels of VEGF are associated with an immunosuppressive tumor microenvironment and diminished response to high-dose IL-2.[2]
- Lucitanib is an investigational antiangiogenic multityrosine kinase inhibitor that targets VEGFR1-3, PDGFRα/β, and FGFR1-3.[3]
- We evaluated the antitumor efficacy and mechanism of action of RDB 1462, the mouse ortholog of ALKS 4230, combined with lucitanib in the MC38 mouse syngeneic colon tumor model.

CONCLUSIONS

- The combination of the intermediate-affinity IL-2R-selective cytokine RDB 1462 and the angiogenesis inhibitor lucitanib resulted in durable dose-dependent antitumor efficacy in the MC38 mouse tumor model.
- Tumor infiltrating immune cells and gene expression changes were consistent with increased antitumor immunity, including increased CD8⁺ T cells and dendritic cells, decreased macrophages, and increased cytolytic gene expression with decreased expression of angiogenesis genes.
- ImmunoSeq analyses demonstrated that T cell receptor diversity increased with RDB 1462 and further increased with the RDB 1462 and lucitanib combination.

METHODS

- C57BL/6 mice were subcutaneously implanted with MC38 cells and, once tumors reached 75 to 125 mm^3, were treated with one of the following:
  - RDB 1462 (1.5 mg/kg sc q3d for 3 weeks) + vehicle (po qd) (n = 10)
  - RDB 1462 (3 mg/kg sc q3d for 3 weeks) + vehicle (po qd) (n = 10)
  - Lucitanib (10 mg/kg po qd for 28 days) + vehicle (sc q3d) (n = 10)
  - RDB 1462 (1.5 mg/kg sc q3d for 3 weeks) + lucitanib (10 mg/kg po qd for 28 days) (n = 10)
  - RDB 1462 (3 mg/kg sc q3d for 3 weeks) + lucitanib (10 mg/kg po qd for 28 days) (n = 10)
  - Vehicle only (sc q3d + po qd) (n = 10)
- Individual mice met study endpoint once tumor volumes reached 1000 mm^3.
- MC38 tumor cell rechallenge experiment:
  - Mice exhibiting a complete response in the first experiment were rechallenged with a second sc injection of MC38 cells on the opposite flank on day 87.
  - 12 treatment-naïve animals were implanted with MC38 cells to confirm malignancy.
- Additional cohorts of mice were treated with the same regimens (until the day before sampling) for analyses on day 7 (n = 5 each), on day 10 (n = 4 each), and on day 16 (n = 5 each).
- Flow cytometric analyses of samples collected on day 10:
  - Single-cell suspensions were made from tumors and spleens, stained for expression of select markers (Table), and acquired on a BD LSRFortessa flow cytometer.
  - Data were analyzed using FlowJo software (Treestar) version 10.0.7r2.
  - Cell populations were plotted as the percentage of CD45⁺ cells.
  - Significance was determined using Dunnett's or Tukey's multiple comparison test.

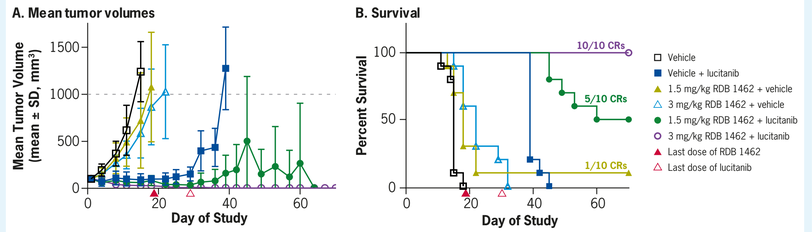
TABLE: Cell Populations and Phenotypic Markers Used for Flow Cytometric Analyses

Cell Population	Phenotypic Markers	Antibody Panel
CD4⁺ T cells	CD45⁺CD11b⁻CD3⁺CD4⁺CD8⁻	CD45, CD3, CD4, CD8, CD11b, CD25, Foxp3, F4/80, CD11c, CD49b, LIVE/DEAD
CD8⁺ T cells	CD45⁺CD11b⁻CD3⁺CD4⁻CD8⁺	
T_{reg} cells	CD45⁺CD11b⁻CD3⁺CD4⁺CD25⁺Foxp3⁺	
Macrophages	CD45⁺CD3⁻CD11b⁺F4/80⁺	
Dendritic cells	CD45⁺CD3⁻CD11b⁻CD11c⁺F4/80⁻	

- RNA-Seq analyses of samples collected on day 10:
  - RNA was isolated from tumors stored in RNAlater using a combination of a Trizol extraction and a Qiagen RNA Mini kit. RNA samples were quantified on a Qubit 4 Fluorometer using the RNA HS assay kit.
  - Gene expression was measured on a ThermoFisher GeneStudio S5 using the Ampliseq mouse Transcriptome kit.
- Gene set enrichment analysis:
  - GSEA[5] assessed enrichment of 167 KEGG pathways (v7.0 MSigDB) in each condition. The heatmap shows 40 most significant GSEA results.
- T cell receptor repertoire analyses of samples collected on days 7 and 16:
  - Analyses were performed on DNA isolated from tumors using the ImmunoSeq platform by Adaptive Biotechnologies.

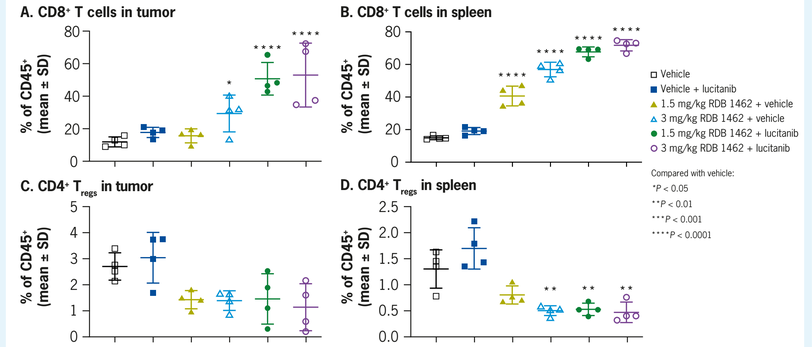
RESULTS

FIGURE 1: Combination of RDB 1462 and Lucitanib Results in Complete Responses and Enhanced Survival Compared With Monotherapy Treatments



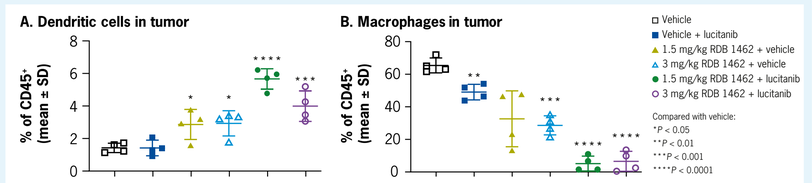
- Both RDB 1462 dose combinations and lucitanib monotherapy resulted in 100% TGI, with TGI rates of 25% and 51% observed following RDB 1462 monotherapy (1.5 and 3 mg/kg, respectively).
- Both dose combinations of RDB 1462 and lucitanib extended survival (MST 68.5 days and > 77 days; low- and high-dose combinations, respectively) versus RDB 1462 (MST 18 and 22 days, respectively) and lucitanib (39 days) monotherapy.
  - All survival curves were significantly different from that for vehicle group (Mantel-Cox, $P < 0.05$).
- Rechallenge of all complete responders (CRs) demonstrated protection mediated by immunological memory (data not shown).

FIGURE 2: RDB 1462, as Monotherapy and in Combination With Lucitanib, Selectively Expands CD8⁺ T Cells in Tumors



- RDB 1462 monotherapy induced significant CD8⁺ T cell expansion in tumor and spleen versus vehicle ($P < 0.05$), with no increase in CD4⁺ T_{regs}.
- The effect of RDB 1462 on CD8⁺ T cells was enhanced when it was given in combination with lucitanib.

FIGURE 3: Combination of RDB 1462 and Lucitanib Resulted in Increased Dendritic Cells but Fewer Tumor-Associated Macrophages Compared With Vehicle

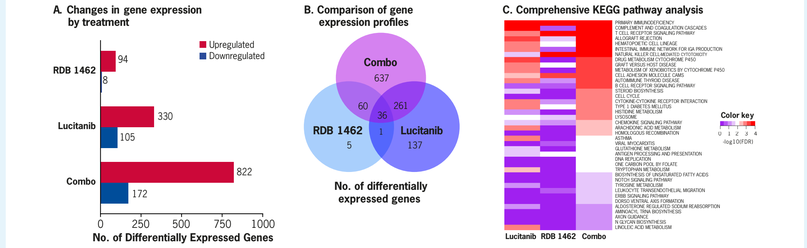


- The combination of RDB 1462 and lucitanib resulted in a significant decrease in macrophages and a significant increase in intratumoral dendritic cells compared to vehicle.

Abbreviations: ANOVA, analysis of variance; *Esm1*, endothelial cell–specific molecule 1; FDR, false discovery rate; FGFR, fibroblast growth factor receptor; GSEA, gene set enrichment analysis; IL-2R, interleukin-2 receptor; KEGG, Kyoto Encyclopedia of Genes and Genomes; MST, mean survival time; PDGFR, platelet-derived growth factor receptor; po, orally; q3d, every 3 days; qd, every day; RPM, reads per million; sc, subcutaneously; SEM, standard error of the mean; TGI, tumor growth inhibition; T_{regs}, regulatory T cells; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

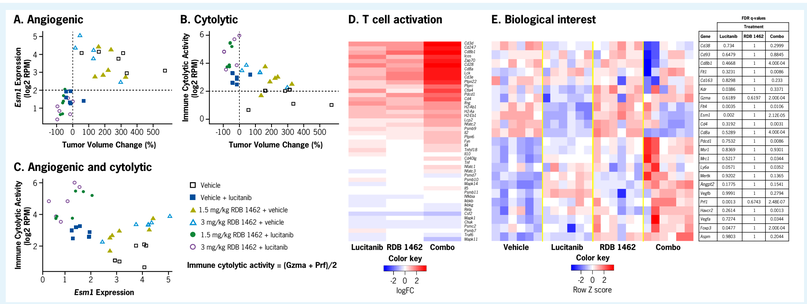
References: 1. Losey HC, et al. Presented at the AACR Annual Meeting; April 1-5, 2017; Washington, DC. Abs 591. **2**. Ott PA, et al. *Front Oncol.* 2015;5:202. **3**. Dusek RL, et al. *Cancer Res.* 2019;79(13 suppl):1214. **4**. Rooney MS, et al. *Cell.* 2015;160:48-61. **5**. Subramanian A, et al. *PNAS* 2005;102(43):15545-15550.

FIGURE 4: Combination of RDB 1462 and Lucitanib Results in Changes in Gene Expression in Tumors Not Observed With Either Monotherapy, as Determined by RNA-Seq Analyses



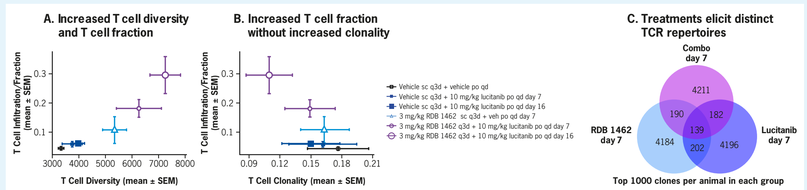
- Genes showing a 2-fold change or greater relative to vehicle ($P < 0.05$; ANOVA) are represented in panels A and B.
- The combination of 3 mg/kg RDB 1462 and lucitanib resulted in an increase in the number of differentially expressed genes (panel A) with a diverse profile (panel B) versus 3 mg/kg RDB 1462 monotherapy or lucitanib monotherapy.
- The heatmap in panel C shows GSEA of KEGG pathways. The 40 most enriched pathways are shown. Color key indicates the negative log10 of *P* values (FDR). Combination treatment resulted in more significant enrichment of pathways associated with monotherapy treatment as well as significant enrichment of unique pathways.

FIGURE 5: Both RDB 1462 and Lucitanib Monotherapy Treatments Induce Cytolytic Gene Expression and T Cell Activation, Which Is Complemented by Lucitanib-Mediated Regulation of VEGF Pathway Genes



- Reduction in expression of *Esm1* (a marker of VEGF activity) correlated with decreased tumor volume in lucitanib mono- and combination therapy–treated animals (panel A).
- RDB 1462 and lucitanib monotherapy induced cytolytic gene expression while combination treatment resulted in greater cytolytic gene expression, which appears to be inversely correlated with changes in tumor volume (panel B).
- Combination treatment resulted in greater cytolytic gene expression as well as reduction in *Esm1* expression (panel C).
- Upregulation of selected genes involved in T cell activation was observed (panel D); color key indicates log2 fold change relative to vehicle.
- Genes with activity in pathways of interest are regulated by combination treatment as indicated by significant q values (FDR; panel E).

FIGURE 6: Combination of RDB 1462 and Lucitanib Results in Increased T Cell Receptor Diversity, Increased T Cell Fraction, and Distinct T Cell Receptor Repertoires in the Tumors of Treated Mice



- T cell fraction, T cell receptor diversity, and Simpson's T cell clonality were determined for tumors collected from mice with the indicated treatments on days 7 and 16. Vehicle-treated and 3 mg/kg RDB 1462 monotherapy groups were excluded from analysis on day 16 due to a number of animals reaching endpoint (Panels A and B).
  - Together, these data suggest the combination of RDB 1462 and lucitanib results in the broadening of the anti-tumor T cell response.
- Top 1000 T cell receptor sequences from each tumor in the indicated groups were compared to evaluate the potential overlap in T cell receptor repertoires (Panel C).

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