First-In-Human Dose Selection for ALKS 4230, an Investigational Immunotherapeutic Agent

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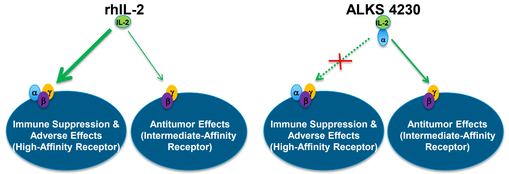
INTRODUCTION

- ALKS 4230 is an engineered fusion protein composed of a circularly permuted interleukin-2 (IL-2) and the IL-2 receptor (IL-2R) α chain, CD25, designed to selectively activate the intermediate-affinity IL-2R, composed of IL-2R β and γ_c.
- The intermediate-affinity IL-2R is expressed predominantly on natural killer (NK) cells and memory CD8 T cells, both of which play an important role in driving antitumor immune responses.[1]
- IL-2 preferentially binds high-affinity IL-2R, composed of IL-2Rα, β, and γ_c, driving the activation and expansion of high-affinity IL-2R-expressing cell types, including immunosuppressive $CD4^+$ regulatory T cells (T_{regs}), which are thought to limit anticancer activity of recombinant human IL-2 (rhIL-2) therapy.[2]
- The high-affinity IL-2R is also expressed on vascular and pulmonary endothelial cells and may contribute to rhIL-2-mediated toxicity via capillary leak syndrome.[3]
- Thus, selective activation of intermediate-affinity IL-2R by ALKS 4230 has the potential to provide enhanced antitumor efficacy as well as improved safety and tolerability.
- Various preclinical *in vitro* and *in vivo* studies were conducted to characterize the pharmacodynamics (PD) and pharmacokinetics (PK) of ALKS 4230; the results guided the dose selection for the ALKS 4230 first-in-human (FIH) clinical study.



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METHODS

- ❑ The following *in vitro* and *in vivo* data were integrated:
  - Concentration vs. STAT5 phosphorylation response in distinct subsets of effector and regulatory lymphocytes from murine, non-human primate (NHP) and human donors was determined using flow cytometry.
  - Concentration vs. cytokine response relationship was determined in an *in vitro* cytokine release assay using fresh human whole blood from healthy donors.
    - ➢ Erbitux® (cetuximab) and Campath® (alemtuzumab) were included in the assay as low and high response comparators, respectively.
    - ➢ Concentrations of 0.01, 0.1, 1.0, or 10 μg/mL were evaluated for each test article (positive controls and ALKS 4230).
    - ➢ Concentrations of IL-2, IL-4, IL-6, IL-8, IL-10, IFNγ and TNFα were measured.
  - Dose and exposure at the No Observed Adverse Effect Level (NOAEL) were determined in an *in vivo* toxicology study in NHP, the most relevant species for toxicology assessment
- ❑ Calculation of human dose to achieve Minimal Anticipated Biological Effect Level (MABEL) or Pharmacologically Active Level (PAL) of circulating ALKS 4230:
  - The EC_{10} for NK cell activation in target cells from human donors, the most sensitive measurement of ALKS 4230 concentrations that induce activation of human IL-2R complex, was used as surrogate for MABEL.
  - The human dose (μg/kg) intended to deliver C_{max} at the MABEL was calculated as (EC_{10} for NK cell activation) x (3 L plasma volume) / (70 kg average body weight)
  - The EC_{50} values for activation of NK cells, memory CD8 T cells and T_{regs} in target cells from human donors were used as surrogates for PAL of ALKS 4230 that induce activation of human IL-2R complex
  - Projected minimal efficacious dose (MED) for a 70 kg human = EC_{50} x 3 L / 70 kg
- ❑ Selection of dose range and escalation scheme for FIH study based on dose and exposure – response comparison:
  - Starting dose = MABEL dose
  - Top dose = human equivalent dose (HED) of NOAEL dose in NHP
  - Half-log dose escalation to bracket projected MED

RESULTS

TABLE 1: Similar *In Vitro* Potency of ALKS 4230 on Human and NHP Lymphocyte Populations, Demonstrate NHP is a Pharmacologically Relevant Species for Toxicology Assessment

In Vitro Potency	NK Cells	CD8 T Cells	T_{regs}
Human			
EC_{10} (nM)	0.09 ± 0.08	0.18 ± 0.06	0.13 ± 0.09
EC_{50} (nM)	0.46 ± 0.08	1.1 ± 0.1	0.59 ± 0.24
NHP			
EC_{10} (nM)	0.10 ± 0.03	0.32 ± .012	0.11 ± 0.03
EC_{50} (nM)	0.48 ± 0.22	1.3 ± 0.4	0.50 ± 0.14
Mouse			
EC_{10} (nM)	5.2 ± 1.6	5.2 ± 0.1	4.8 ± 2.9
EC_{50} (nM)	12 ± 4	17 ± 2	11 ± 5

Data presented are mean ± standard deviation values generated from three experiments with each sample being stimulated in triplicate.

TABLE 2: Selection of Dose Range and Escalation Scheme for FIH Study Based on Dose/Exposure – Response Comparison

Approach	Surrogate Measurement	Human Dose
MABEL Dose	EC_{10} for activation of human NK cells: 0.0031 μg/mL (0.09 nM)	0.1 μg/kg
MED	EC_{50} for activation of human NK cells: 0.016 (0.46 nM) CD8 T cells: 0.038 (1.1 nM)	0.7 μg/kg 1.6 μg/kg
HED of NOAEL in NHP	NOAEL of 100 μg/kg in NHP	32 μg/kg

- Based on the comparison of MABEL dose (0.1 μg/kg) to MED (0.7– 1.6 μg/kg) and HED of NOAEL in NHP (32 μg/kg), the proposed doses to be evaluated in the FIH study are 0.1, 0.3, 1, 3, 10 and 30 μg/kg.

TABLE 3: Concentration - Dependent Increases in IL-6, IL-8, and IFN-γ Were Observed in Majority of Donor Samples Treated With ALKS 4230 in an *In Vitro* Cytokine Release Assay

Cytokine Levels (pg/mL)	Erbitux (μg/mL)				Campath (μg/mL)				ALKS 4230 (μg/mL)			
	10	1	0.1	0.01	10	1	0.1	0.01	10	1	0.1	0.01
IL-2	0	0	0	0	0	0	0	0	0	0	0	0
IL-4	0	0	0	0	0	0	0	0	0	0	0	0
IL-6	0	0	0	0	943	864	613	0	1130	971	335	0
IL-8	0	0	0	0	246	172	136	0	1064	880	357	0
IL-10	0	0	0	0	0	0	0	0	0	0	0	0
IFN-γ	0	0	0	0	349	351	336	0	1404	528	0	0
TNF-α	0	0	0	0	0	0	0	0	0	0	0	0

Data presented are median cytokine concentrations (pg/mL) measured in human whole blood samples from 20 healthy donors.

TABLE 4: MABEL Dose is Considered a Safe Starting Dose for the FIH Study Based on Exposure Comparison

	Ratio to Projected C_{max} at MABEL Dose in Human
C_{max} at NOAEL in NHP 2.43 (M) - 2.61 (F) μg/mL	784 - 842
Lowest Concentration in Cytokine Release Assay 0.01 μg/mL	3

Projected C_{max} at MABEL dose in human is 0.0031 μg/mL, the in vitro EC_{10} for NK cell activation, assuming IV administration to a 70 kg human with 3 L plasma volume.

DISCLOSURES

This study was funded by Alkermes, Inc.
Lei Sun, Heather C. Losey and Lisa von Moltke are employees of Alkermes, Inc. Juan C. Alvarez is a former employee of Alkermes, Inc. William J Slichenmyer is a contractor of Alkermes, Inc.

CONCLUSIONS

- The starting dose for the FIH study was chosen based on MABEL, a conservative approach to mitigate potential risk in targeting IL-2R, a high risk target.
- Preclinical PK, PD and toxicology assessments support the FIH investigation of ALKS 4230 at the proposed dose range and escalation scheme.

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