Characterization of the Pharmacodynamic Immune Response to a Novel Immunotherapeutic Agent, ALKS 4230, in Mice and Non-Human Primates

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ABSTRACT

ALKS 4230 is a selective agonist of the intermediate-affinity IL-2 receptor (IL-2R). A phase 1 study is ongoing to evaluate the safety and tolerability of ALKS 4230 in the treatment of patients with refractory solid tumors. The selectivity of ALKS 4230 is achieved through the stable fusion of circularly permuted IL-2 to the extracellular portion of the IL-2R α chain, CD25. The resulting fusion protein is sterically prevented from binding to the high-affinity IL-2R complex, comprised of IL-2Rα, IL-2Rβ, and common gamma chain, expressed preferentially on CD4$^+$ FOXP3$^+$ regulatory T cells (CD4$^+$ T_{regs}) yet retains full ability to signal through the intermediate-affinity IL-2R complex, comprised of IL-2Rβ and common gamma chain, expressed on memory CD8$^+$ T cells and NK cells. Repeated dosing of ALKS 4230 drives the significant expansion of various CD8$^+$ T cell and NK cell populations without activation and minimal expansion of CD4$^+$ T_{regs} in mice and non-human primates (NHP). The kinetics of the immunological responses in mice and NHP demonstrate that the pharmacodynamic effects persist beyond systemic exposure of ALKS 4230.

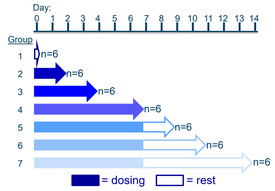
METHODS

Pharmacokinetic Analysis in Mice

- 8-10 week old male C57BL/6 mice were given a single administration of 3 µg ALKS 4230 IV or 8 µg ALKS 4230 SC.

Pharmacodynamic Analysis in Mice

- 8-10 week old male C57BL/6 mice were dosed subcutaneously with 16 µg ALKS 4230 QD for the indicated number of days.
- One day after the last dose, animals were euthanized, and spleens were harvested and weighed.
- Splenocytes were isolated by homogenization and RBC lysis, surface-stained with different panels of antibodies to identify various immune populations, fixed/permeabilized, and stained intracellularly for Foxp3.



Pharmacokinetic Analysis in Cynomolgus Monkeys

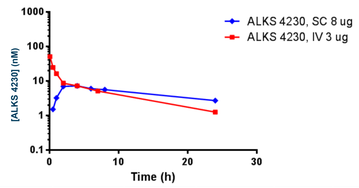
- Male drug naïve cynomolgus monkeys were given a single IV administration of ALKS 4230 at 0.03 mg/kg, 0.1 mg/kg, and 0.3 mg/kg (n = 3 per dose level).

Pharmacodynamic Analysis in Cynomolgus Monkeys

- 3 Male drug naïve cynomolgus monkeys were given 0.1 mg/kg ALKS 4230 IV QD for 5 days (days 0-4).
- Peripheral whole blood was collected prior to the first dose and the dose on day 3, and then subsequently on days 6-10, 14 and 21.
- RBCs were lysed, remaining cells were stained with different panels of antibodies to identify various immune populations, fixed/permeabilized, and stained intracellularly for FoxP3.

RESULTS

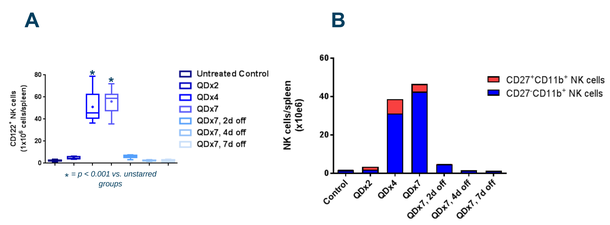
FIGURE 1: QD dosing of ALKS 4230 in C57BL/6 mice is expected to achieve circulating drug levels sufficient to induce cellular expansion



Dosing Route	Dose (µg)	C_{max} (nM)	T_{max} (h)	$AUC_{0\text{-last}}$ (nM*h)	$t_{1/2}$ (h)	$MRT_{0\text{-last}}$ (h)
IV	3	54.2	0.083	140	8.0	5.6
SC	8	7.65	4	119	15.4	9.6

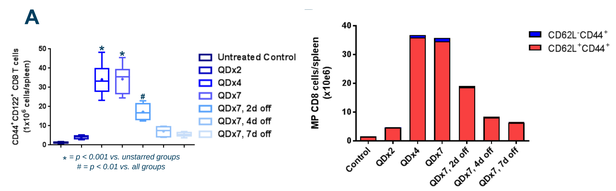
- ALKS 4230 EC_{50s} for *in vitro* STAT5 phosphorylation in CD122+ NK cells and memory phenotype CD8 T cells are 12 nM and 17 nM, respectively
- QD dosing of 16 µg ALKS 4230 administered SC is expected to achieve circulating drug levels sufficient to drive cellular expansion

FIGURE 2: ALKS 4230 rapidly expands CD27$^-$CD11b$^+$ NK cell numbers in C57BL/6 mice after 4 days of QD dosing



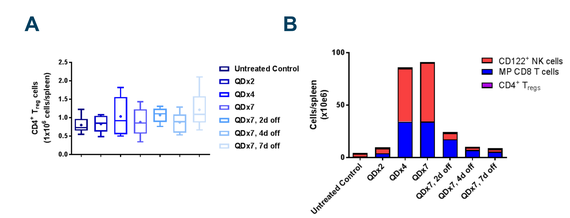
C57Bl/6 mice were dosed with ALKS 4230 QD and then rested for the indicated lengths of time prior to tissue harvest and analysis (n = 6 per group). (A) CD122$^+$ NK cell numbers are increased in spleens after 4 days of dosing; but decrease as early as 2 days after cessation of treatment (statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparisons test). (B) ALKS 4230 expands CD27$^-$CD11b$^+$ NK cells, suggesting a fully differentiated/cytotoxic phenotype.

FIGURE 3: ALKS 4230 expands CD8 T cells with a central memory phenotype in C57BL/6 mice after 4 days of QD dosing



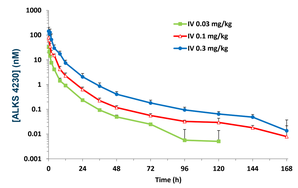
(A) CD44$^+$CD122$^+$ CD8 T cell numbers are dramatically increased in the spleens of C57Bl/6 mice dosed with ALKS 4230 QD (statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparisons test). (B) Expanded CD8 T cells exhibit a CD62L$^+$CD44$^+$ central memory-like phenotype.

FIGURE 4: ALKS 4230 selectively expands NK cells and MP CD8 T cells over CD4$^+$Foxp3$^+$ T_{regs} in C57BL/6 mice



(A) Total numbers of CD4$^+$CD25$^+$CD127^{low}Foxp3$^+$ cells (CD4$^+$ T_{regs}) were quantified in the spleens of mice dosed with ALKS 4230 or untreated controls. ALKS 4230 does not induce the expansion of CD4$^+$ T_{regs}.(B) NK cells and MP CD8 T cells are expanded after 4 days of dosing with ALKS 4230 while CD4$^+$ T_{reg} numbers do not change.

FIGURE 5: ALKS 4230 displays a dose-dependent increase in systemic exposure in cynomolgus monkeys after a single IV administration

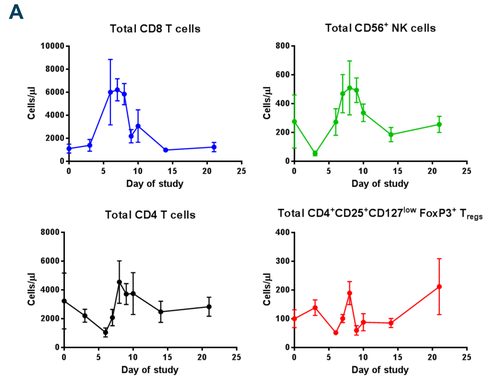


Dose (mg/kg)	C_{max} (nM)	$AUC_{0-\infty}$ (nM*h)	$t_{1/2}$ (h)	MRT (h)	Vd_{ss} (mL/kg)	CL (mL/h/kg)
0.03	32.7 ± 3.2	73.1 ± 3.6	20.2 ± 8.3	6.7 ± 1.0	79.7 ± 8.1	11.9 ±0.6
0.1	79.7 ± 12.5	204 ± 22	34.0 ± 8.7	7.5 ± 1.4	107 ± 16	14.4 ± 1.7
0.3	155 ± 60	564 ± 177	32.7 ± 6.5	8.2 ± 0.7	138 ± 49	16.6 ± 5.4

* Data presented are the mean +/- SD (n=3)

- ALKS 4230 EC_{50s} for *in vitro* STAT5 phosphorylation in NK cells and CD8 T cells are 0.5 nM and 1.5 nM, respectively
- QD dosing of 0.1 mg/kg ALKS 4230 administered IV is expected to achieve circulating drug levels sufficient to drive cellular expansion

FIGURE 6: 0.1 mg/kg ALKS 4230 dosed QDx5 in cynomolgus monkeys induces the selective expansion of CD8 T cells and CD56$^+$ NK cells over CD4$^+$ T_{regs}



Select immune cell populations in peripheral blood were identified by flow cytometry and quantified relative to counting beads. (A) Numbers of total CD4 T cells, total CD8 T cells, total CD56$^+$ NK cells, and CD4$^+$CD25$^+$CD127^{low}FoxP3$^+$ T_{reg} cells. Data plotted are the mean +/- SD. (B) Fold change comparison of CD8 T cells, CD56$^+$ NK cells, and CD4$^+$ T_{regs} reveals that ALKS 4230 preferentially expands CD8 T cells and CD56$^+$ NK cells. Preferential expansion results in increased ratios of CD8 T cells/CD4$^+$ T_{regs} (C) and CD56$^+$ NK cells/ CD4$^+$ T_{regs} (D). Data plotted in B-D are the mean +/- SEM.

SUMMARY

- ALKS 4230 in C57BL/6 mice:
  - QD dosing achieves systemic exposure sufficient to induce lymphocyte expansion
  - Induces the expansion of CD27$^-$CD11b$^+$ cytotoxic NK cells and CD44$^+$CD62L$^+$ MP CD8 T cells after only 4 days of dosing
  - Does not induce an increase in CD4$^+$$T_{reg}$ cell numbers
  - Expanded NK cell population decreases 2 days after dosing has ceased while expanded MP CD8 T cell population decreases at a slower rate
- ALKS 4230 in cynomolgus monkeys:
  - Exhibits a dose-dependent increase in systemic exposure after a single IV administration
  - Induces the expansion of CD8 T cells and CD56$^+$ NK cells but not CD4$^+$ T cells or CD4$^+$ T_{regs}
  - Selective expansion results in increased ratios of CD8 T:CD4$^+$ T_{reg} and CD56$^+$ NK:CD4$^+$ T_{reg} cells

CONCLUSIONS

- ALKS 4230 induces dose-dependent selective expansion of memory CD8 T cells and cytotoxic NK cells in mice and non-human primates.
- The pharmacodynamic effects induced by ALKS 4230, namely the expansion of subsets of CD8 T cells and NK cells, are observed after several days of drug exposure; these PD effects continue to be observed for several days after the drug has cleared from circulation.
- ALKS 4230 is being tested in an ongoing phase 1 study to evaluate safety and tolerability in the treatment of patients with refractory solid tumors.

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