Predicting a human efficacious dose range for entolimod (CBLB502) based on biomarkers of its anti-radiation efficacy

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Abstract

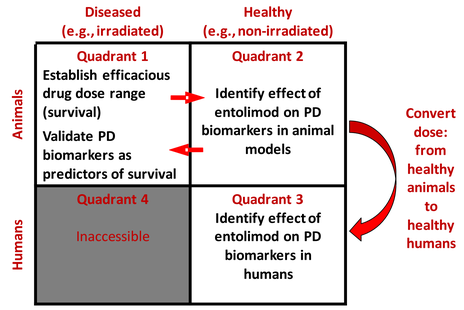
Introduction: Protection of the human organism from ionizing radiation and treatment of the acute radiation syndrome (ARS) are the key problems in biodefense, nuclear safety, radiation therapy, and space travel. Entolimod (CBLB502) is a Cleveland BioLabs, Inc proprietary candidate medical radiation countermeasure (MRC) that acts via interaction with Toll-like receptor 5 and subsequent induction of multiple protective and mitigating mechanisms[1]. Entolimod was shown to be an effective MRC in both rodent and non-human primate (NHP) models: a single administration increased survival by at least 30-50% when administered within -24 to +48 hours relative to LD50-LD90 doses of radiation.

Methods: Since efficacy testing of MRC against lethal ARS in humans is ethically impossible, entolimod is developed under Food and Drug Administration (FDA) Animal Rule. Thus, its efficacious dose range in humans must be estimated indirectly based on animal studies, e.g. using efficacy biomarkers[2]. Levels of multiple factors were measured in blood of non-irradiated animals and healthy human subjects to identify candidate biomarkers responding to entolimod in a dose-dependent manner[3]. The association between the entolimod dose response of these biomarkers and survival was next evaluated in irradiated animals. Finally, we applied statistical modeling to dose responses of biomarker effects in non-irradiated animals and human subjects to estimate the target efficacious dose range in humans.

Results: We identified a number of cytokine- and blood parameter-based biomarkers of entolimod efficacy that respond to entolimod treatment in a dose-dependent manner in irradiated and non-irradiated animals of multiple species, as well as in healthy human subjects. At least two of these biomarkers (G-CSF, IL-6) demonstrated direct involvement in anti-radiation survival efficacy of entolimod (as per FDA requirements). In GLP/GCP entolimod survival efficacy study (in 179 NHP exposed to 70% lethal total body irradiation and treated 25 hours later), these biomarkers displayed a close predictive association with the anti-radiation efficacy of entolimod. The efficacious entolimod dose range in NHP was defined, with full efficacy observed at ≥10 µg/kg, showing survival increase from 27.5% in placebo to 70-75%. Statistical model-based comparison of biomarker responses to entolimod in healthy humans vs. those of healthy NHP (from GLP pharmacokinetics/ pharmacodynamics study in 160 NHP) predicted efficacious entolimod dose range in human subjects starting at ~0.4 µg/kg (~30-35 µg/subject)..

Conclusions: To satisfy the requirements of FDA's Animal Rule, we developed an approach to predict the efficacious dose range of entolimod in humans, based on biomarker responses to entolimod in animals and human subjects and its anti-radiation efficacy in animals. Using the data from clinical studies and GLP studies in NHP model, an approximate efficacious dose range was estimated for entolimod in humans. Licensure of this potent MRC will facilitate protection of military personnel operating in contaminated environments, as well as treatment of victims in radiation emergencies.

Dose conversion framework



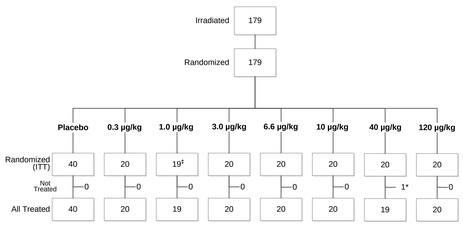
Must reach biomarker levels in humans that are equal to those induced in animals by efficacious drug doses.

Biomarkers for entolimod dose conversion:

G-CSF, IL-6, ANC (neutrophil count)

- **Increase with entolimod dose in animals and humans; follow survival in animals; mediators of drug's anti-radiation efficacy in animals[3].**
- **Samples are easy to obtain, assays available**

GLP/GCP survival/PD/PK study of entolimod efficacy in NHPs



Goal: to evaluate effect of entolimod (given at 25 hours after LD70 TBI in a range of doses) on survival, hematological parameters, and biomarker responses in NHP model.

[Quadrant 1]

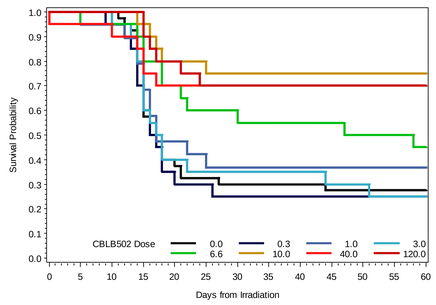
Endpoints: 60-day survival, platelet and neutrophil counts, biomarker responses

Supportive care: minimal (analgesics, nutrition, but no antibiotics or blood products).

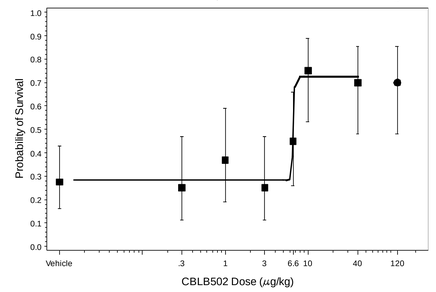
GLP study with elements of GCP (randomization, blinding, statistical plan, robust data collection)

Survival efficacy of entolimod in NHPs 25 h after $LD_{70/60}$ TBI

Kaplan-Meier survival curves (60-day)



Dose dependence of survival efficacy



- **Entolimod treatment raises 60-day survival from 27.5% in control to 70-75% at 10-120 µg/kg doses**
- **P<0.002 for trend for increased survival at 10-120 µg/kg (P<0.0024 by Fisher's exact text)**
- **10 µg/kg is the optimal CBLB502 dose**
- **Timing of moribundity/mortality is generally similar for control and entolimod-treated groups**

Entolimod effects on efficacy biomarkers

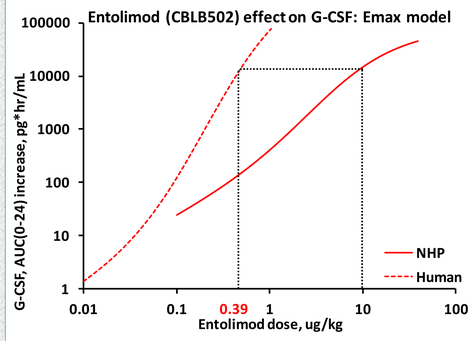
Correlations between dose-dependent effects of entolimod on survival and efficacy biomarker responses in *irradiated* NHP [Quadrant 1]

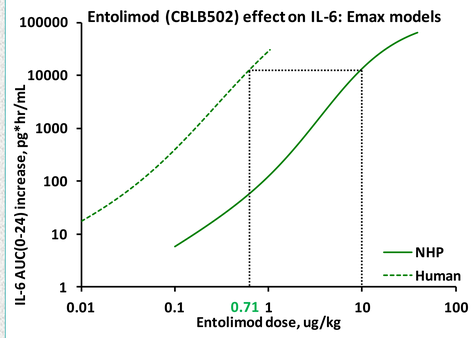
Biomarker	Spearman Rank Correlation
G-CSF	0.84
IL-6	0.84
ANC24	0.84

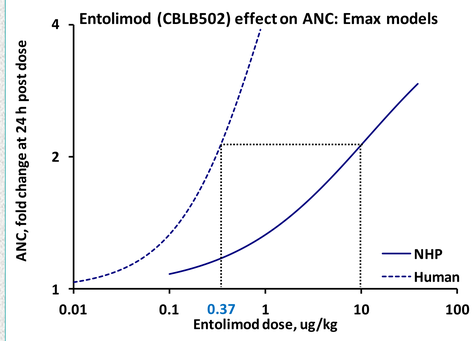
- **Biomarkers increase with dose: G-CSF, IL-6 – monotonous/ Emax, ANC – bell-shaped response**
- **These increases are statistically significant (P<.0001 for trend tests)**
- **High correlation for effects of CBLB502 on all 3 biomarkers and its effects on survival, with similar plateau/peak location: ~10-40 µg/kg**

Results of dose conversion

Biomarker dose response models in non-irradiated NHPs and humans [Quadrants 2, 3]







Similar human doses are predicted by entolimod efficacy biomarkers:

G-CSF: 0.39 µg/kg
IL-6: 0.71 µg/kg*
ANC: 0.37 µg/kg

* Higher dose predicted by IL-6 is likely to be assay-related

Summary

1. **A framework for biomarker-based efficacious dose conversion between species has been developed by CBLI**
2. **Statistically robust GLP/GCP study of entolimod survival efficacy and biomarker effects has been completed in NHP model of lethal ARS**
3. **Close association (r = 0.84) of biomarkers (G-CSF, IL-6 and ANC) with post-TBI survival was confirmed**
4. **Entolimod treatment increased survival after $LD_{70/60}$ TBI in a dose dependent manner, from 27.5% for placebo to 70-75% for fully efficacious doses**
5. **Entolimod dose of 10 µg/kg was identified as optimal efficacious dose**
6. **Using biomarker data from non-irradiated NHPs and healthy humans, dose conversion indicated human entolimod doses of ~0.4-0.6 µg/kg as equivalent to 10 µg/kg NHP dose and, thus, potentially efficacious in treatment of human ARS**

References

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2. US Food and Drug Administration Animal Efficacy Rule, 21CFR601.91(a)
3. Krivokrysenko V.I., Shakhov A., Singh V.K. et al. (2012). "Identification of G-CSF and IL-6 as Candidate Biomarkers of CBLB502 Efficacy as a Medical Radiation Countermeasure." J Pharmacol Exp Ther. 2012 Jul 26

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