EOS100850 inhibits A_{2A} receptor signaling in human whole blood: two pharmacodynamic assays to monitor EOS100850 activity in clinical studies

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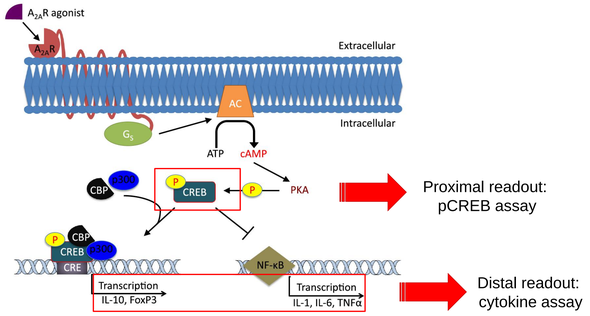
BACKGROUND

- High levels of extracellular adenosine promote immune suppression
- A_{2A} receptor is the most prevalent adenosine receptor of immune cells
- Adenosine signaling through A_{2A} receptor suppresses innate and adaptive immune reactions, in particular antitumor immunity
- In the tumor microenvironment, adenosine concentrations are > 10-fold higher than in the normal counterpart
- iTeos A_{2A} receptor antagonist EOS100850 is designed for immuno-oncology:
  - ✓ Selective inhibitor of A_{2A} receptor
  - ✓ Potent regardless of the adenosine levels, in particular in high adenosine concentrations
  - ✓ Non-brain penetrant, avoiding potential CNS side-effects at doses needed to inhibit tumoral A_{2A} receptor
  - ✓ Rescues adenosine-driven T cell and innate cell immunosuppression
- iTeos A_{2A} receptor antagonist EOS100850 is in the clinic:
  - ✓ Phase 1/1B clinical study ongoing (FPFV in February 2019)
  - ✓ Two pharmacodynamic assays used to monitor target engagement in blood from dosed patients

A_{2A} RECEPTOR SIGNALING PATHWAY



Adapted from: Jones KR, Kang EM – Comput Struct Biotechnol J (2015)

PROXIMAL READOUT OF A_{2A} RECEPTOR ENGAGEMENT: pCREB ASSAY

Principle of the assay:

- In peripheral blood lymphocytes:
  - the A_{2A} receptor agonist CGS-21680 (CGS) induces CREB phosphorylation (pCREB) via PKA
  - phorbol 12-myristate 13-acetate and ionomycin (PMA) induce pCREB via PKC
- EOS100850 inhibits pCREB induced via A_{2A} receptor but not by the unspecific stimulant PMA

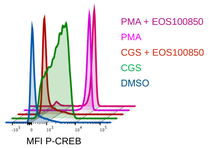


Fig. 1: Induction and inhibition of pCREB.
Whole blood was *ex vivo* stimulated with DMSO (blue histogram), or CGS-21680 (green and red histograms), or PMA (pink and violet histograms) in the presence or in the absence of 1 µM EOS100850. pCREB was analyzed by flow cytometry. Staining of pCREB on gated $CD4^+$ cells is shown for one representative donor.

CREB IS PHOSPHORYLATED AFTER STIMULATION WITH CGS-21680 IN HEALTHY AND CANCER DONORS

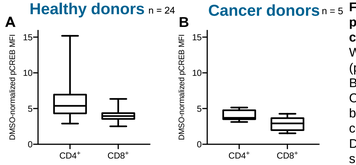


Fig. 2: pCREB is induced in peripheral blood $CD4^+$ and $CD8^+$ cells.
Whole blood of 24 healthy donors (panel A) and 5 cancer donors (panel B) was stimulated with DMSO or CGS-21680. pCREB was analyzed by flow cytometry in CD4+ and $CD8^+$ cells.
DMSO-normalized pCREB MFI are shown as with Tukey plots

pCREB ASSAY IS REPEATABLE

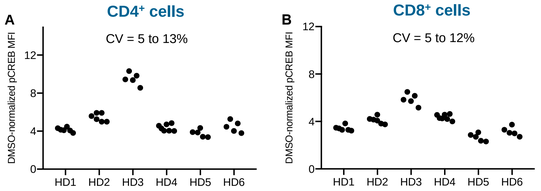


Fig. 3: pCREB assay is repeatable.
Whole blood of 6 healthy donors was stimulated with DMSO or CGS-21680. The assay was repeated in 5-7 independent runs (sample stimulation, staining, acquisition). pCREB was analyzed by flow cytometry in $CD4^+$ (panel A) and $CD8^+$ (panel B) cells.
Each dot represents one run. DMSO-normalized pCREB MFI are shown. HD: Healthy donor.

CREB PHOSPHORYLATION INDUCED BY CGS-21680 IS INHIBITED BY EOS100850 IN A DOSE-DEPENDENT MANNER

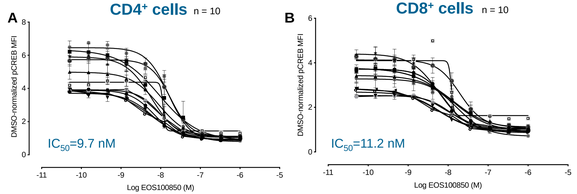


Fig. 4: pCREB is inhibited by EOS100850 in peripheral blood lymphocytes.
Whole blood of 10 healthy donors was *ex vivo* incubated with increasing doses of EOS100850, followed by stimulation with DMSO or CGS-21680 for 45 minutes. pCREB was analyzed by flow cytometry in $CD4^+$ (panel A) and $CD8^+$ (panel B) cells.
Each line represent one donor. DMSO-normalized pCREB MFI are shown as mean ± SD.

pCREB CAN BE ANALYZED ON STIMULATED AND CRYOPRESERVED BLOOD SAMPLES

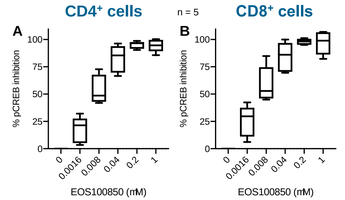


Fig. 5: pCREB is inhibited by EOS100850 in peripheral blood lymphocytes of cancer donors.
Whole blood of 5 cancer donors was incubated with increasing doses of EOS100850, followed by stimulation with DMSO or CGS-21680. pCREB was analyzed by flow cytometry in $CD4^+$ (panel A) and $CD8^+$ (panel B) cells of cryopreserved samples.
DMSO-normalized pCREB MFI are shown with Tukey plots.

CRYOPRESERVED SAMPLES ARE STABLE FOR AT LEAST SIX MONTHS WHEN STORED AT -80°C

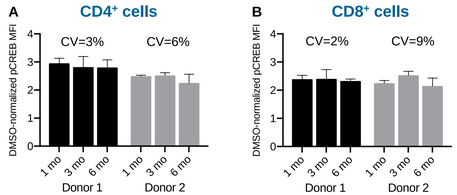


Fig. 6: Stability of stimulated and cryopreserved blood samples.
Whole blood of 2 healthy donors was stimulated with DMSO or CGS-21680. pCREB was analyzed by flow cytometry in $CD4^+$ (panel A) and $CD8^+$ (panel B) cells of cryopreserved samples 1-3-6 months after freezing.
DMSO-normalized pCREB MFI are shown as mean ± SD, together with the coefficient of variation (CV).

DISTAL READOUT OF A_{2A} RECEPTOR ENGAGEMENT: CYTOKINE ASSAY

Principle of the assay:

- In peripheral blood, LPS stimulates secretion of cytokines and chemokines
- The A_{2A} receptor agonist CGS-21680 (CGS) alters LPS-stimulated secretion of cytokines and chemokines
- EOS100850 restores CGS-induced alterations of cytokine and chemokine secretion in LPS-stimulated blood
- Customized TruCulture tubes (Myriad RBM) allow monitoring of EOS100850 activity

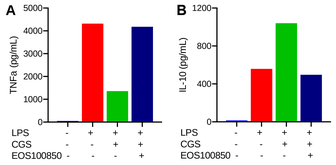


Fig. 7: Induction and inhibition of cytokine secretion.
Whole blood was *ex vivo* stimulated with LPS (red histogram), or LPS + CGS-21680 ± 1 µM EOS100850 (green and blue histograms) using TruCulture tubes (Myriad RBM). TNF-α (panel A) and IL-10 (panel B) were quantified in frozen culture supernatants using Luminex. Data from one representative donor.

CYTOKINE SECRETION IS RESTORED BY EOS100850 IN A DOSE-DEPENDENT MANNER

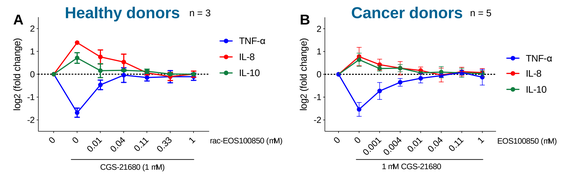


Fig. 8: EOS100850 reverts CGS-induced alterations of cytokine secretion in LPS-stimulated blood.
Whole blood of 3 healthy donors (panel A) and 5 cancer donors (panel B) was stimulated with LPS alone or LPS and CGS-21680 in the presence of increasing doses of EOS100850 using TruCulture tubes (Myriad RBM). Levels of TNF-α, IL-8 and IL-10 were analyzed in culture supernatants. Data are shown as mean ± SD.

CONCLUSIONS

The pharmacodynamic activity of EOS100850 in clinical trials is monitored in blood using two qualified assays:

- Excellent precision and stability
- Suitable for analysis of cancer patients
- Minimal volume of drawn blood (< 3 mL total)
- Minimal sample manipulation in the clinical sites
- Optimized for analysis of cryopreserved samples

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