2. MAb screening and lead selection

MAbs identified via Adimab’s human B-cell cloning platform using RSV pre-F protein as bait.

Screening of yeast mAbs using RSV-A2 wild-type virus

Expression of the most potent mAbs (top 10%) and comparator mAbs in CHO cells

In vitro characterization and benchmarking in different assay formats: sequence optimization

Nomination of 2 lead candidates based on in vitro potency analyses for in vivo testing

3. Binding competition studies with selected leads

Lead mAb #1, site Ø
- Epitope region shared with D25/MEDI8897* but epitope differs based on binding competition studies and epitope mapping
- REGN2222 competes with MEDI8897*, but not with lead mAb #1
- Lead mAb #1 binds to a linear peptide within site Ø, while MEDI8897* does not

Lead mAb #2, site IV
- Weak competition with 101F4
- Lead mAb #2 does not bind to linear peptides of the site IV region

4. Comparative in vitro neutralization potency

ELISA based Microneutralization Assay

qPCR

Focus Inhibition Assay

CHO expressed mAbs were tested in multiple assay formats and compared to known, well-characterized anti-RSV mAbs. Examples with RSV-prototype strains in different assay formats are shown.

5. In vivo efficacy testing in cotton rat model

Animals immunized i.m. with different mAb doses, 24 hours prior to intranasal challenge with RSV-A2 or RSV-B/Wash; viral load determined by plaque assay

- Lead mAb #1 was clearly more potent than palivizumab, REGN2222 or R81 and protective at 1 and 3 mg/kg doses, similarly to MEDI8897*.
- Site IV binders had lower efficacy in reducing the viral load in the nose.

6. Selection of escape mutants

RSV-A2 passaged on Hep-2 cells in presence of neutralizing mAbs

- Palivizumab led to selection of a known mutation5 in passaging round 2 (K272M, site II).
- Site IV lead mAb #2 lost protection in round 3 due to a mutation in site IV, Glycine 446 (change to V and E, respectively).
- No escape mutants identified for antigenic site Ø lead mAb #1 and MEDI8897*.

7. Conclusions & Outlook

- 2 highly potent anti-RSV mAbs were selected that bind to the pre-fusion form of RSV-F and broadly neutralize subtype A and B strains.
- Lead mAb #1 targets antigenic site Ø (pre-F specific), while lead mAb #2 is binding to antigenic site IV (accessible on both the pre- and post-fusion form of RSV-F).
- The two mAbs exhibit approximately 50-100 fold improvement in potency relative to Synagis and display at least comparable, but up to 10-fold higher virus neutralization activity than the most potent comparator mAb MEDI8897* in RSV strain dependent fashion.
- Lead mAb #1 was superior over palivizumab, REGN2222 and R81 in a cotton rat RSV-A2 challenge model and the efficacy was comparable to that of MEDI8897*.
- Lead mAb #2 had weaker efficacy in controlling RSV replication in the nose.

Escape mutant selection studies suggested that site Ø is less prone to pressure by neutralizing mAbs, unlike site II and IV.