Superior Efficacy of Ozoralizumab, an anti-Human TNF Nanobody in a TNF Transgenic Mouse Model of Polyarthritis

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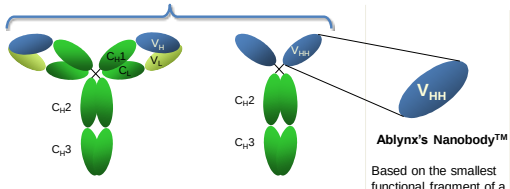
Disclosures:

Martin Hegen, Marina Shen, Julie Lee, Lori Fitz, Yulia Vugmeyster, Christopher Wrocklage, Nilufer Seth, Kyri Dunussi-Joannopoulos, Cheryl Nickerson-Nutter and Mary Collins are employees of Pfizer Inc.

Els Beirnaert, Guy Hermans and Peter Casteels are employees of Ablynx nv and hold stock in Ablynx nv.

Nanobody: The Smallest Functional Fragment of a Naturally occurring Heavy-chain Antibody

Camelidae family has both forms



Conventional Antibody

Heavy and light chains
Both chains required for antigen binding and stability

Heavy-Chain Antibody

Only heavy chains
Full antigen binding capacity and very stable

Ablynx's Nanobody™

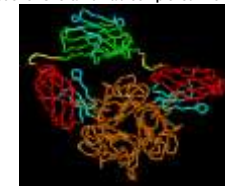
Based on the smallest functional fragment of a naturally occurring heavy-chain antibody

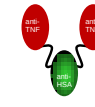
Revets H. et al. *Exp Opin Biol Ther* 2005, 5:111-24.

Ozoralizumab (ATN-103): Humanized, Trivalent, Bi-specific Nanobody

- **Novel humanized therapeutic protein derived from camelid heavy-chain antibody**
  - 38 kDa protein, one quarter the size of Adalimumab, Infliximab or Etanercept
- **Two anti-TNF Nanobody building blocks**
  - Expected potency comparable with Etanercept
  - Humanized llama domain: 98% homology versus human
- **One anti-HSA Nanobody building block**
  - Half-life extension
  - Humanized llama domain: 92% homology versus human

Model of Ozoralizumab complexed with TNF





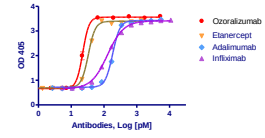
Binding affinities of Ozoralizumab and TNF inhibitors to TNF

TNF inhibitor	*Human TNF Kd (pM)*	*Rhesus TNF Kd (pM)*
Ozoralizumab	20.2	16.1
Etanercept	11.6	24.2
Adalimumab	23.7	25.8
Infliximab	22.7	No binding

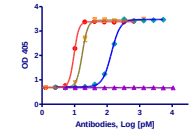
Ozoralizumab demonstrated identical equilibrium dissociation constants (*Kd*) based on binding to human and rhesus monkey albumin.

Ozoralizumab shows Comparable Potency to Etanercept and Superior Potency to Adalimumab and Infliximab in L929 Cell Based Assay

Human TNF

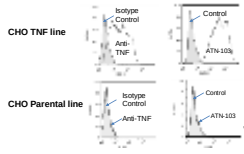


Rhesus TNF

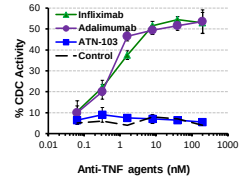


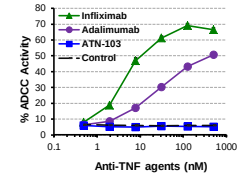
EC50, pM	Ozoralizumab	Etanercept	Adalimumab	Infliximab
Human TNF	22.5	33.3	184	128.5
Rhesus TNF	9.6	17.3	135	No response

Flow Cytometry demonstrating Binding of Ozoralizumab to Cell Surface TNF

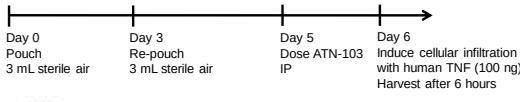


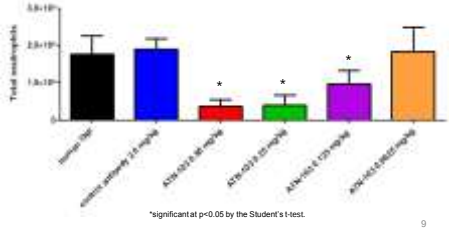
Ozoralizumab (ATN-103) has no Observed Complement-dependent Cytotoxicity (CDC) or Antibody-dependent Cell-mediated Cytotoxicity (ADCC) Activity





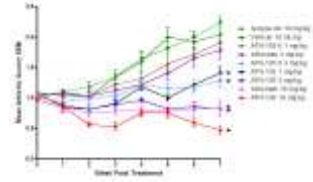
Effect of Ozoralizumab (ATN-103) on Neutrophil Infiltration





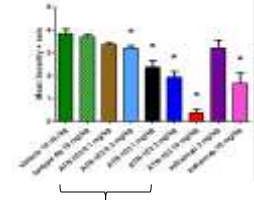
*significant at p<0.05 by the Student's t-test.

Ozoralizumab shows Superior Efficacy in the Human TNF Transgenic Tg197 Mouse Model of Arthritis



Human TNF transgenic Tg197 mouse

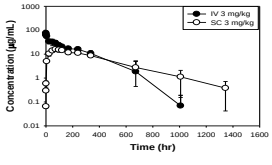
Biomedcode Hellas SA.

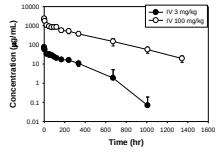


Ozoralizumab (ATN-103)

Histopathological score:
0: No detectable pathology
1: Hyperplasia of the synovial membrane and presence of polymorphonuclear infiltrates
2: Pannus and fibrous tissue formation and focal subchondral bone erosion
3: Cartilage destruction and bone erosion
4: Extensive cartilage destruction and bone erosion

PK Profiles of Ozoralizumab after a single IV or SC Dose to Cynomolgus Monkeys





- The mean half-life after a single IV or SC dose ranged from 7-10 days
- The bioavailability after a single SC dose was ~70%

Pharmacokinetic analysis in mice and in non-human primates confirmed the long *in vivo* half-life of Ozoralizumab

Conclusions:

- **Ozoralizumab has high affinity for human TNF and showed equivalent potency on a molar basis as compared to Etanercept in a cell based assay**
- **Ozoralizumab has no observed complement-dependent cytotoxicity (CDC) or antibody-dependent cell-mediated cytotoxicity (ADCC) activity**
- **In comparison with Infliximab, Ozoralizumab demonstrated superior efficacy in a therapeutic treatment protocol in the human TNF transgenic mouse model of arthritis**
- **Pharmacokinetic analysis showed long *in vivo* half-life of Ozoralizumab in non-human primates**

Please note:

- **Pharmacokinetic-Pharmacodynamic Modeling of Ozoralizumab (ATN-103), a Novel Humanized Nanobody TNF Inhibitor for Rheumatoid Arthritis**
  Poster Presentation **#125:** Rheumatoid Arthritis Treatment - Small Molecules, Biologics, Therapy:
  Monday, November 7, 9:00 AM - 6:00 PM

- **A Multiple Ascending Dose / Proof of Concept study of ATN-103 (ozoralizumab) in Rheumatoid Arthritis Subjects on a background of Methotrexate**
  Oral Presentation #2630: Rheumatoid Arthritis Treatment - Small Molecules, Biologics, Therapy: Novel Compounds II
  Wednesday, November 9, 11:00 AM - 12:30 PM
  Presentation Time: **11:45 AM - 12:00 PM** Room W 375 A