The development and characterization of NM26-2198, an anti-IL-4Rα anti-IL-31 bispecific antibody to reduce inflammatory immune activation and pruritus in patients with moderate to severe atopic dermatitis

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Concept:

NM26-2198 is a bispecific antibody in an IgG4-scFv fusion format which targets IL-4Rα (type I and type II receptors) and IL-31 in a bivalent manner. NM26-2198 is designed to prevent IL-4 and IL-31-induced keratinocyte immunopathology, immune cell activation, barrier defects and pruritus (itch phenotype) in atopic dermatitis through the concomitant inhibition of IL-4/IL-13 signaling via the blockade of the IL-4R and neutralization of IL-31. NM26-2198 binds to IL-4R on hematopoietic cells and keratinocytes and potently blocks the pro-inflammatory IL-4/IL-4R signaling axis. IL-31 blockade prevents inflammatory responses via macrophages and keratinocytes as well as inhibiting pruritus through a direct effect on sensory nerves as a key factor in halting the feedback loop of skin barrier damage. NM26-2198 is therefore designed to provide a faster onset of efficacy and a potential larger effect size through the concomitant modulation of both pathways.

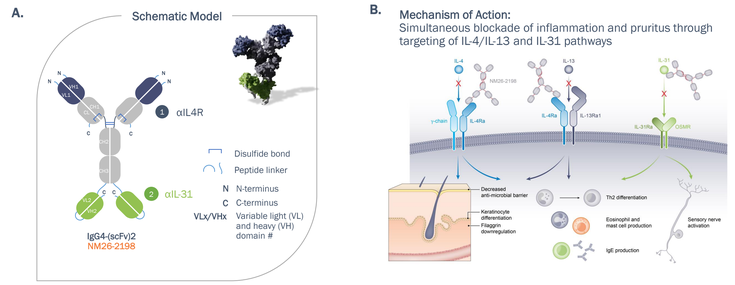


Figure 1. **A.** Schematic representation and structural model of NM26-2198, a bispecific and bivalent IgG4-(scFv)2 fusion molecule targeting IL-4Rα and IL-31. Two λ-capped anti-IL-31 scFv are fused to the C-termini of the anti-IL4Rα IgG4 heavy chain via flexible peptide linkers. **B.** Interleukin (IL)-4, IL-13 and IL-31 are key cytokines involved in the pathophysiology of atopic dermatitis (AD). IL-4 is also responsible for B cell activation and IgE mediated responses, while IL-31 is mainly involved in pruritus. Through concomitant blockade of the IL-4/IL-4R signaling axis and IL-31 induced signaling, NM26-2198 has the potential to reduce both the inflammatory immune activation and itch, leading to improved disease pathology and quality of life in patients.

NM26-2198 binds with high affinity to IL-4Rα and IL-31

A.

Target	ka (1/Ms)	kd (1/s)	KD (M)
IL-4Rα	3.14E+05	3.84E-05	1.21E-10
IL-31	2.58E+05	1.95E-05	7.56E-11

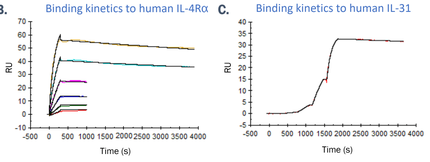


Figure 2. **A. Binding kinetics to IL-4Rα and IL-31.** NM26-2198 binds to IL-4Rα and IL-31 with picomolar affinity. Binding kinetics were determined by SPR analysis on a T200 Biacore instrument using capture assays. Representative SPR sensorgrams illustrating binding kinetics to human IL-4Rα using multicycle kinetics (**B**) and single cycle binding kinetics to human IL-31 (**C**). The colored lines represent the experimental data while the black lines show the fit of a 1:1 interaction model to the data.

NM26-2198 potently blocks IL-4R, with and without concomitant engagement with IL-31

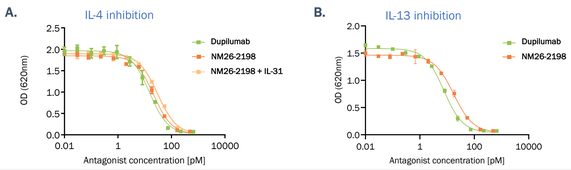


Figure 3. Blockade of human IL-4 and IL-13 induced IL-4R signaling in a STAT-6 reporter gene assay using HEK-Blue™ cells. The inhibition of the IL-4R pathway leads to decreased secretion of embryonic alkaline phosphatase, resulting in lower absorbance values. Dupilumab* was used as a comparator molecule. **A.** NM26-2198 potently blocks IL-4 induced signaling and no loss of potency is observed in the presence of an excess quantity of IL-31. **B.** NM26-2198 also potently blocks IL-13 induced IL-4R signaling.

NM26-2198 potently blocks IL-31, with and without concomitant engagement with IL-4R

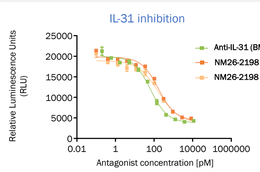


Figure 4. Blockade of IL-31 induced signaling via the heterodimerization of human IL-31RA and OSMR beta in osteosarcoma cells. The inhibition of human IL-31 induced receptor heterodimerization in this reporter cell line leads to decreased production of active beta-galactosidase by the cells, resulting in a decrease of luminescent signals. The inhibitory potency of NM26-2198 was assessed, using the anti-IL-31 antibody BMS-981164* as comparator molecule. NM26-2198 potently blocks IL-31 and the blockade is maintained in presence of an excess quantity of IL-4R.

NM26-2198 potently inhibits IL-4 induced CD23 expression on human monocytes and naïve and memory B cells

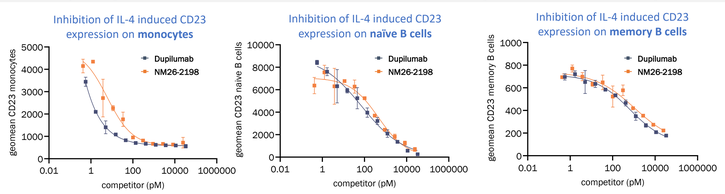
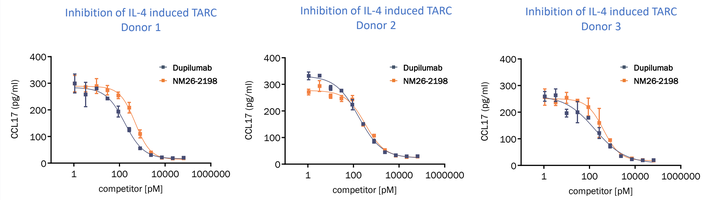


Figure 5. Blockade of human IL-4 induced upregulation of CD23 by NM26-2198 in a cell-based assay using human PBMC subsets. CD23 (low affinity IgE receptor) expression was measured by flow cytometry on human monocytes, naïve B cells and memory B cells following isolation of PBMC from fresh human blood. Isolated PBMCs were incubated for 48 hours at 37°C in presence of a constant concentration of IL-4, serial dilutions of NM26-2198 and dupilumab*. The cells were then stained for flow cytometry analysis and the geometric mean of gated CD23 monocytes, CD23 naïve B cells and CD23 memory B cells was used for calculations. The assay was performed with fresh blood from 4 donors. One representative experiment is shown in this figure. As compared to dupilumab*, NM26-2198 inhibits IL-4 induced CD23 expression with similar potency in naïve and memory B cells and with slightly lower potency in monocytes.

NM26-2198 inhibits IL-4 induced secretion of TARC (CCL17) in whole blood with similar potency as dupilumab

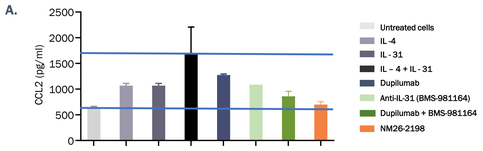


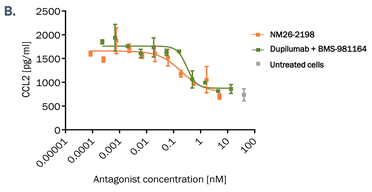
NM26-2198	IC_{50}	Relative IC_{50}
Blood donor	[pM]	(IC90 dupilumab/IC90 NM26-2198)
1	2700	0.52
2	2908	0.77
3	2250	1.93
Mean IC_{50}	2619	1.07
STDEV IC_{50}	336.3	0.75

Figure 6. Blockade of human IL-4 induced thymus and activation regulated chemokine (TARC) in human blood. Whole human peripheral EDTA blood was incubated for 24 hours at 37°C with 1 ng/ml human IL-4 and serial dilutions of NM26-2198 or dupilumab*. TARC concentration in cell supernatant was then quantified by ELISA.
NM26-2198 inhibited TARC secretion with similar potency as dupilumab*. IC_{90} values are donor dependent and ranged from 2250 to 2908 pM with an average IC_{90} value of 2619 ± 336.3 pM.

NM26-2198 simultaneously blocks the effects of IL-4Rα and IL-31 signaling

Blockade of IL-4 and IL-31 induced activation of bronchial epithelial cells





- ✓ NM26-2198 concomitantly blocks the IL-4R and IL-31RA pathways
- ✓ The combined inhibition by NM26-2198 is more efficacious than the single blockade of either IL-4R or IL-31 pathway alone

Figure 7. Blockade of human IL-4 and human IL-31 induced activation of Bronchial Epithelial Cells. Human bronchial epithelial cells (BEAS-2B) express both type I and type II IL-4R. They also express low levels of IL-31RA. Stimulation of cells with human IL-31 or human IL-4 individually induces the release of CCL2. The combination of IL-4 and IL-31 causes an additive stimulation of CCL2 secretion.

BEAS-2B cells were incubated for 24 hours at 37°C with 1 ng/ml IL-4, 10 ng/ml IL-31 and with serial titrations of NM26-2198, dupilumab*, the anti-IL-31 antibody BMS-981164* or a combination of dupilumab* and BMS-981164*. Cell supernatants were collected following incubation and released CCL2 was quantified using a sandwich ELISA. **A.** CCL2 release in the presence of the IL-4, IL-31 and the selected antagonists at 1 µg/ml each (5 nM dupilumab and 5 nM BMS-981164* for the combination and 6.7 nM NM26-2198). **B.** CCL2 release in the presence of IL-4 and IL-31 and a dose titration of NM26-2198 or a combination of dupilumab and BMS-981164*.

NM26-2198 treatment of healthy skin biopsies reduces the number of genes significantly dysregulated by IL-4+IL-13+IL-31

A.

Cytokine used for treatment	Number of dysregulated genes
IL-4 + IL-13	499
IL-31	64
IL-4 + IL-13 + IL-31	1702

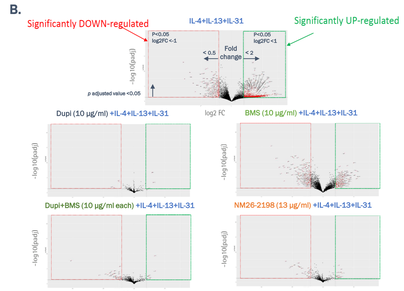
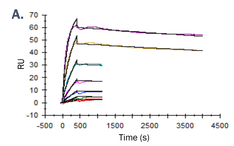
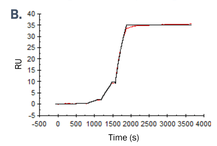


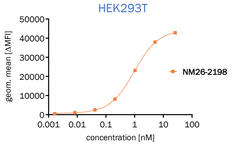
Figure 8. Investigation of the effect of NM26-2198 to prevent IL-4, IL-13 and IL-31 induced pathology of normal human skin biopsies.
A. Normal human skin punch tissue samples were collected from consenting donors and treated with cytokines. IL-4 and IL-13 treatment induced a robust dysregulation of 499 genes, which was further exacerbated by the addition of IL-31, resulting in 1702 dysregulated genes. IL-31 treatment alone induced the dysregulation of 64 genes only. This suggests that IL-31 increases IL-4 and IL-13 dependent changes in gene expression in a synergistic way (raw data not shown).
B. The skin biopsies were treated with IL-4, IL-13 and IL-31 at 50 ng/ml each in the presence or absence of NM26-2198, dupilumab*, the anti-IL-31 antibody BMS-981164* and a control IgG. All antibodies were used at a concentration of 67 nM each. RNA-seq analysis was performed and the data was summarized as volcano plots, showing fold-changes of up-and downregulation of genes (with p-adjusted log10 values on the y axis). The addition of NM26-2198, dupilumab or dupilumab+BMS-981164* led to a significant reduction in the number of up-and down-regulated genes mediated by IL-4+IL-13+IL-31 treatment of the skin punches, with >98% inhibition of gene expression changes. The addition of BMS-981164* alone had a limited impact on the observed gene dysregulation.

- ✓ NM26-2198 has a similar effect than the combination of dupilumab and BMS-981164* in inhibition of IL-4+IL-13+IL-31 induced gene expression changes

NM26-2198 is cross-reactive with cynomolgus monkey IL-4Rα and IL-31





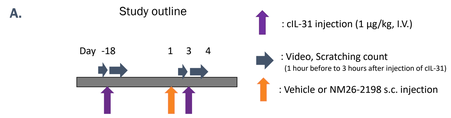


D.

Binding kinetics			
Target	ka (1/Ms)	kd (1/s)	KD (M)
Cyno IL-4Rα	2.88E+05	3.54E-05	1.23E-10
Cyno IL-31	8.32E+04	<1.00E-05	1.20E-10
EC50 for binding to IL-4Rα on HEK293T cells (nM)			
Cyno IL-4Rα	0.97		

Figure 9. **A.** NM26-2198 binds to cynomolgus monkey IL-4Rα with a high affinity of 123 pM as determined by multicycle kinetic SPR analysis using a T200 Biacore instrument. **B.** A KD of 120 pM was determined for NM26-2198 binding to cynomolgus IL-31 by single cycle SPR analysis. **C.** NM26-2198 also bound to IL-4Rα expressed on the surface of HEK293T cells transiently transfected with a plasmid encoding for cynomolgus IL-4Rα. NM26-2198 was incubated with the cells for 24 hours and binding to cynomolgus IL-4Rα was determined by flow cytometry. **D.** Summary table of the binding kinetics and affinity by SPR towards cynomolgus IL-4Rα and IL-31 and EC_{50} value determined for binding to cynomolgus IL-4Rα expressed on HEK293T cells.

NM26-2198 subcutaneous administration reduces IL-31 induced pruritus and scratching behavior in cynomolgus monkeys in a dose-dependent manner



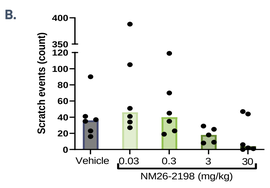


Figure 10. Effect of NM26-2198 on IL-31 induced pruritus in cynomolgus monkeys. **A.** Cynomolgus monkey IL-31 (cIL-31)-induced scratching was recorded in male monkeys on day -18 and the animals were randomized to treatment groups, excluding animals with strong baseline scratching behavior. On day 1, NM26-2198 was administered subcutaneously at 0 (control), 0.03, 0.3, 3 and 30 mg/kg to the monkeys. 48 hours later, cIL-31 was injected intravenously at a dose of 1 µg/kg. The frequency of scratching behavior was evaluated for 1 hour pre-injection of cIL-31 and for 3 hours post-injection of cIL-31.
B. Decreases in the frequency of scratching were observed in monkeys dosed with 3 and 30 mg/kg of NM26-2198 with a significant decrease at 30 mg/kg compared to the control group (by multiple comparison using ordinary log-transformed values with William's test).

Single intravenous or subcutaneous injections of up to 125 mg/kg of NM26-2198 show expected pharmacokinetic profile in non-human primates

- ✓ A PK study was conducted in cynomolgus monkey with single doses of NM26-2198 administered intravenously at 1, 5 and 25 mg/kg and subcutaneously at 5, 25 and 125 mg/kg
- ✓ Non-linear pharmacokinetics most likely due to target mediated drug disposition to IL-4R
- ✓ Mean half lives of between 7 and 10 days were determined following i.v. or s.c. administration, similar to dupilumab surrogate REGN646 (dupilumab is not cross-reactive to cynomolgus IL-4R)

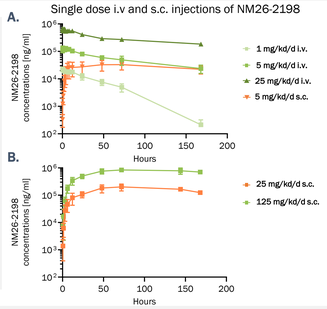


Figure 11. Pharmacokinetics in cynomolgus monkeys. **A.** NM26-2198 was administered i.v. to monkeys at 1, 5 and 25 mg/kg/day and s.c. at 5 mg/kg/day **B.** NM26-2198 was injected s.c. at 25 and 125 mg/kg/day.

NM26-2198 is well tolerated in cynomolgus monkeys with weekly s.c. administration at 125 mg/kg

- ✓ A formal 4-week repeat dose GLP toxicology study in cynomolgus monkeys was conducted
- ✓ 5 weekly repeat doses of NM26-2198 were administered
  - ✓ s.c. @ 5, 25 and 125 mg/kg
  - ✓ i.v. @ 125 mg/kg
- ✓ NM26-2198 was well tolerated in all groups
  - ✓ No test-item related clinical observations
  - ✓ No gross pathological findings
  - ✓ No histopathological findings
  - ✓ No site injection inflammation
  - ✓ No ophthalmology findings
  - ✓ No cardiovascular, respiratory or neurological findings observed
- ✓ No Observed Effect Level (NOEL) in cynomolgus monkeys is 125 mg/kg, based on the highest dose tested

Conclusions

- ✓ **NM26-2198 is a bispecific antibody with dual antagonistic activity to inhibit IL-4R-driven inflammation through IL-4 and IL-13 and to inhibit IL-31 induced inflammation and pruritus**
- ✓ **Reporter cell and primary cell assays demonstrate the potent ability of NM26-2198 to concomitantly block IL-4R and IL-31 pathways**
- ✓ **Gene dysregulation induced by treatment of healthy skin biopsies with IL-4, IL-13 and IL-31 is efficiently reduced by NM26-2198**
- ✓ **NM26-2198 is cross-reactive to cynomolgus IL-4R and cynomolgus IL-31**
- ✓ **In an IL-31 induced pruritus cynomolgus model, scratching frequency was significantly reduced in monkeys treated with NM26-2198**
- ✓ **NM26-2198 has expected pharmacokinetic properties, with mean $t_{1/2}$ in the range of 7 to 10 days in monkeys**
- ✓ **Multiple dose administrations to cynomolgus monkey were well tolerated in a 4-week GLP toxicology study with NOEL at 125 mg/kg, the highest dose tested**
- ✓ **NM26-2198 is a promising therapeutic candidate with excellent PK/PD properties and a favorable safety profile in healthy monkeys**
- ✓ **A FIH SAD study is planned to be performed in healthy volunteers, followed by a MAD study in patients with moderate to severe atopic dermatitis**

Acknowledgments

- ✓ **Thanks to the team at Monasterium Laboratories for their scientific input and expertise in performing the skin biopsy experiments (Fig. 8)**

* Dupilumab and BMS-981164 was generated at Numab Therapeutics based upon published sequence information