

# The development and characterization of NM26-2198, an anti-IL-4R $\alpha$ 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-4R $\alpha$  (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 potentially blocks the pro-inflammatory IL-4/IL-13 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.

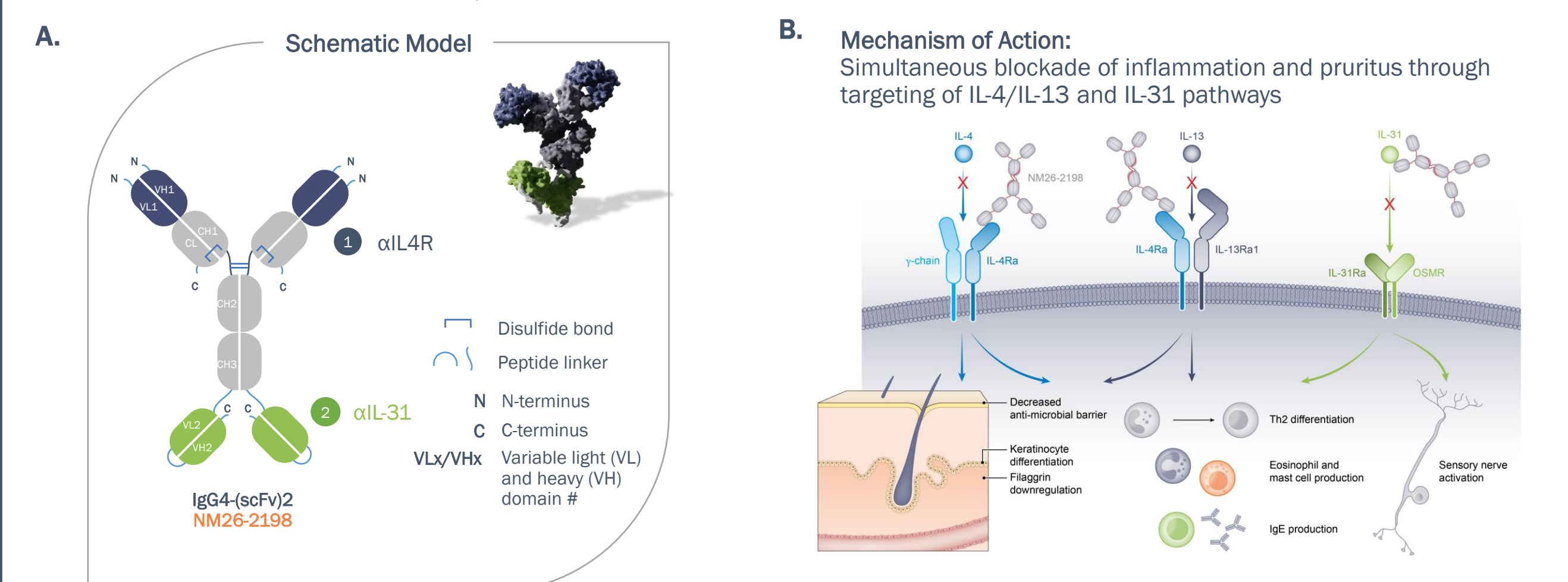
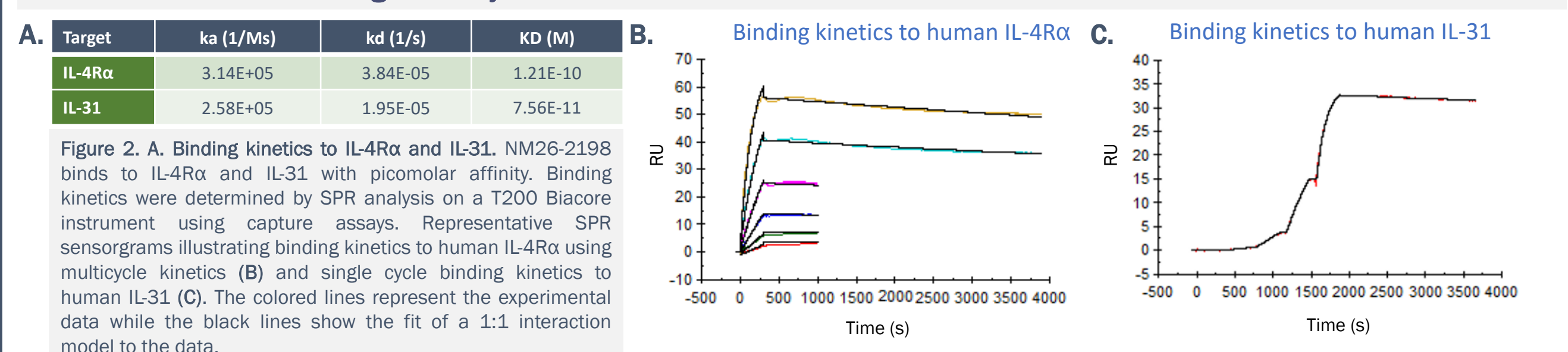


Figure 1. A. Schematic representation and structural model of NM26-2198, a bispecific and bivalent IgG4-(scFv)<sub>2</sub> fusion molecule targeting IL-4R $\alpha$  and IL-31. Two A-capped anti-IL-31 scFvs are fused to the C-termini of the anti-IL-4R $\alpha$  IgG4 heavy chain via flexible peptide linkers. 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-13 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-4R $\alpha$ and IL-31



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

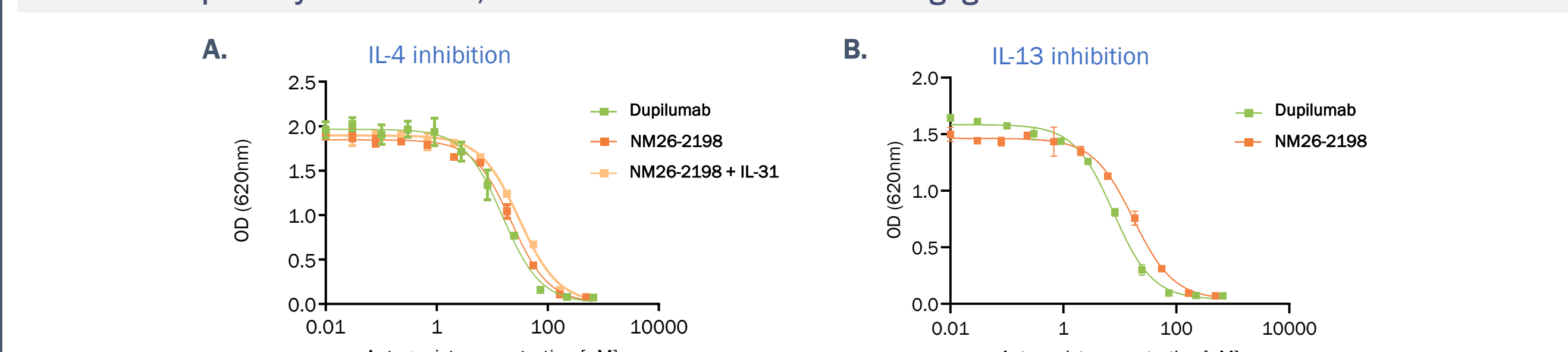
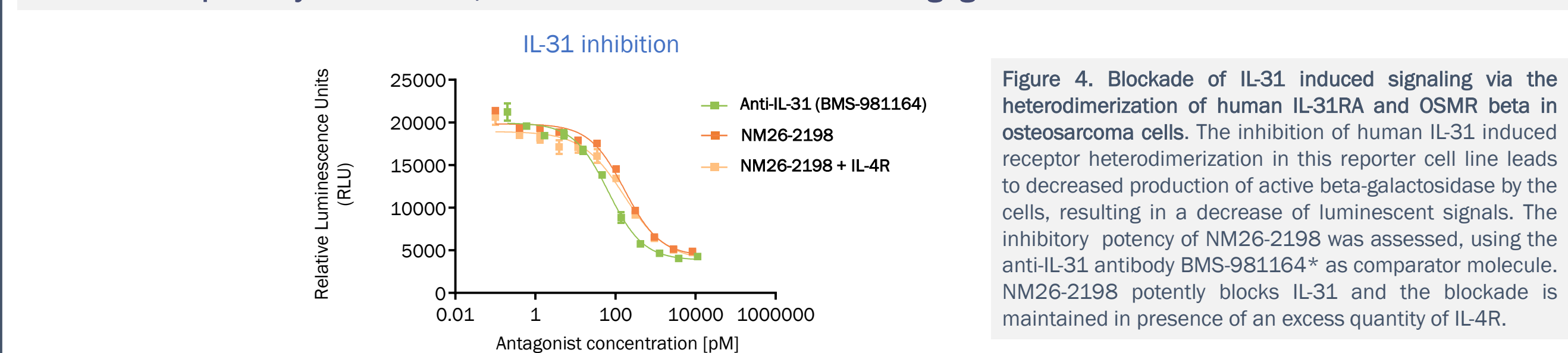


Figure 3. Blockade of human IL-4 and IL-13 induced IL-4R signaling in a STAT-6 reporter gene assay using HEK-Blue™ cells. The inhibition of the IL-4R pathway leads to decreased secretion of embryonic alkaline phosphatase, resulting in lower absorbance values. Dupilumab™ was used as a comparator molecule. 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



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

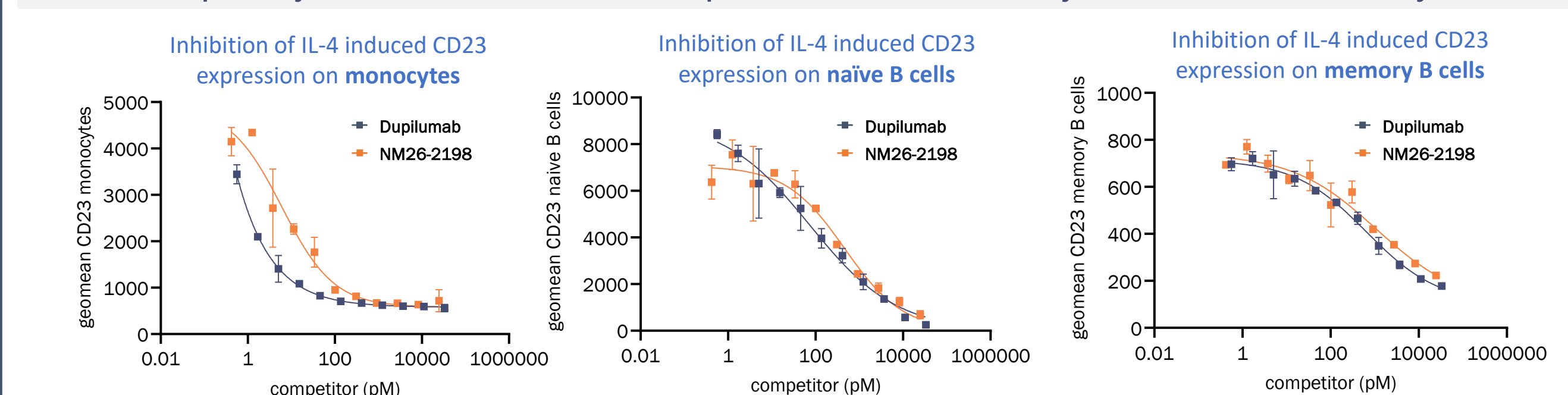


Figure 5. Blockade of human IL-4 induced upregulation of CD23 by NM26-2198 in a cell-based assay using human PBMC subsets. CD23 (low affinity IgE receptor) expression was measured by flow cytometry on human monocytes, naïve B cells and memory B cells following isolation of PBMC from fresh human blood. 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

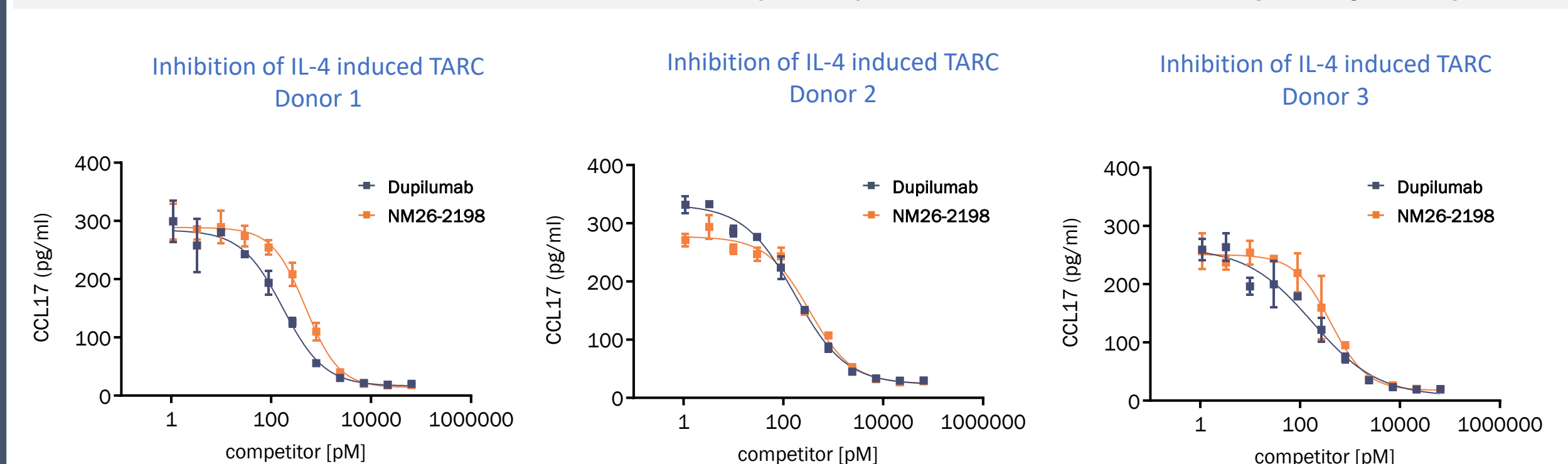


Figure 6. Blockade of human IL-4 induced thymus and activation regulated chemokine (TARC) in human blood. Whole human peripheral EDTA blood was incubated for 24 hours at 37°C with 1 ng/ml human IL-4 and serial dilutions of NM26-2198 or dupilumab™. TARC concentration in cell supernatant was then quantified by ELISA. NM26-2198 inhibited TARC secretion with similar potency as dupilumab™. IC<sub>50</sub> values are donor dependent and ranged from 2250 to 2908 pM with an average IC<sub>50</sub> value of 2619 ± 336.3 pM.

## NM26-2198 simultaneously blocks the effects of IL-4R $\alpha$ and IL-31 signaling

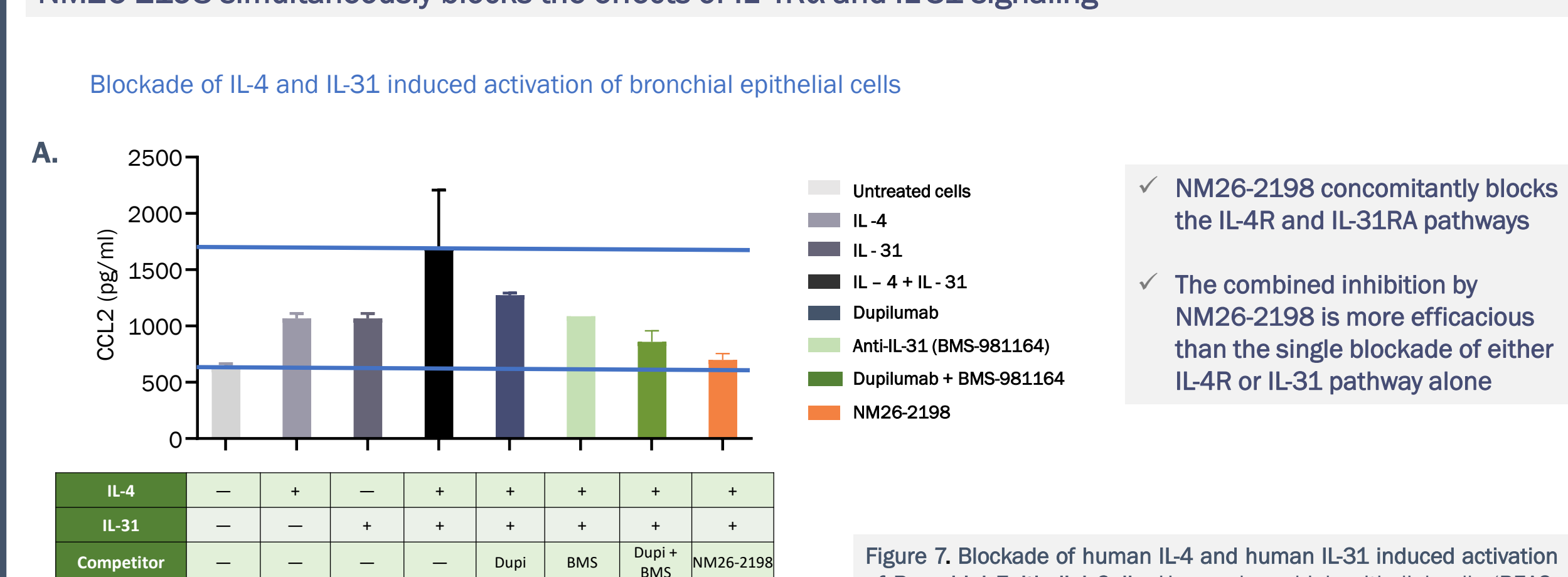


Figure 7. Blockade of human IL-4 and human IL-31 induced activation of Bronchial Epithelial Cells. Human bronchial epithelial cells (BEAS-2B) express both type I and type II IL-4R. They also express low levels of IL-31RA. Stimulation of cells with human IL-31 or human IL-4 individually induces the release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

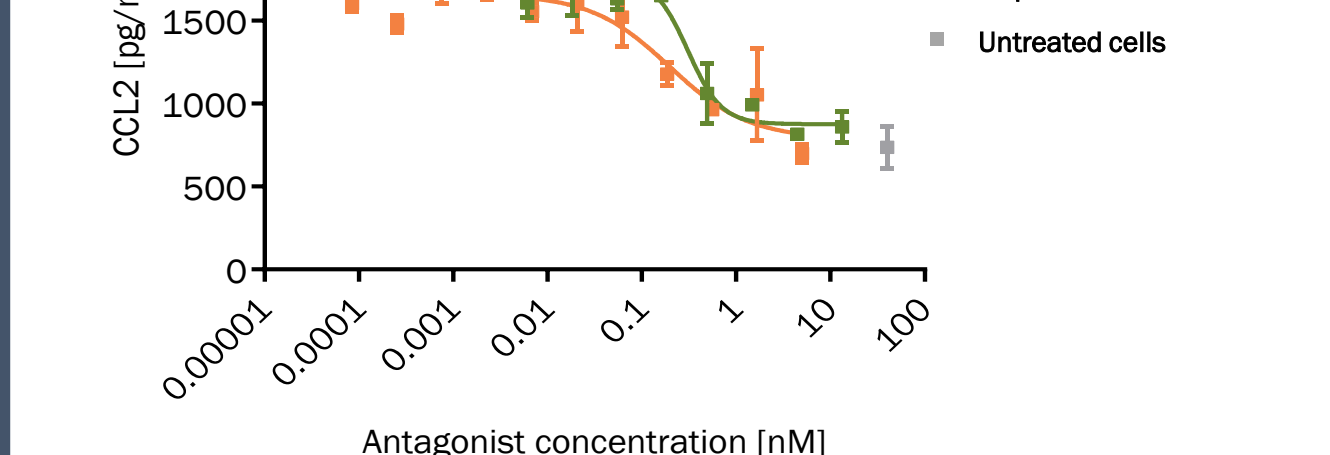


Figure 8. Investigation of the effect of NM26-2198 to prevent IL-4, IL-13 and IL-31 induced pathology of normal human skin biopsies. A. Normal human skin punch tissue samples were collected from consenting donors and treated with cytokines. IL-4 and IL-13 treatment induced a robust dysregulation of 499 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 down-regulation of genes (with padjusted 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 treatment of healthy skin biopsies reduces the number of genes significantly dysregulated by IL-4+IL-13+IL-31

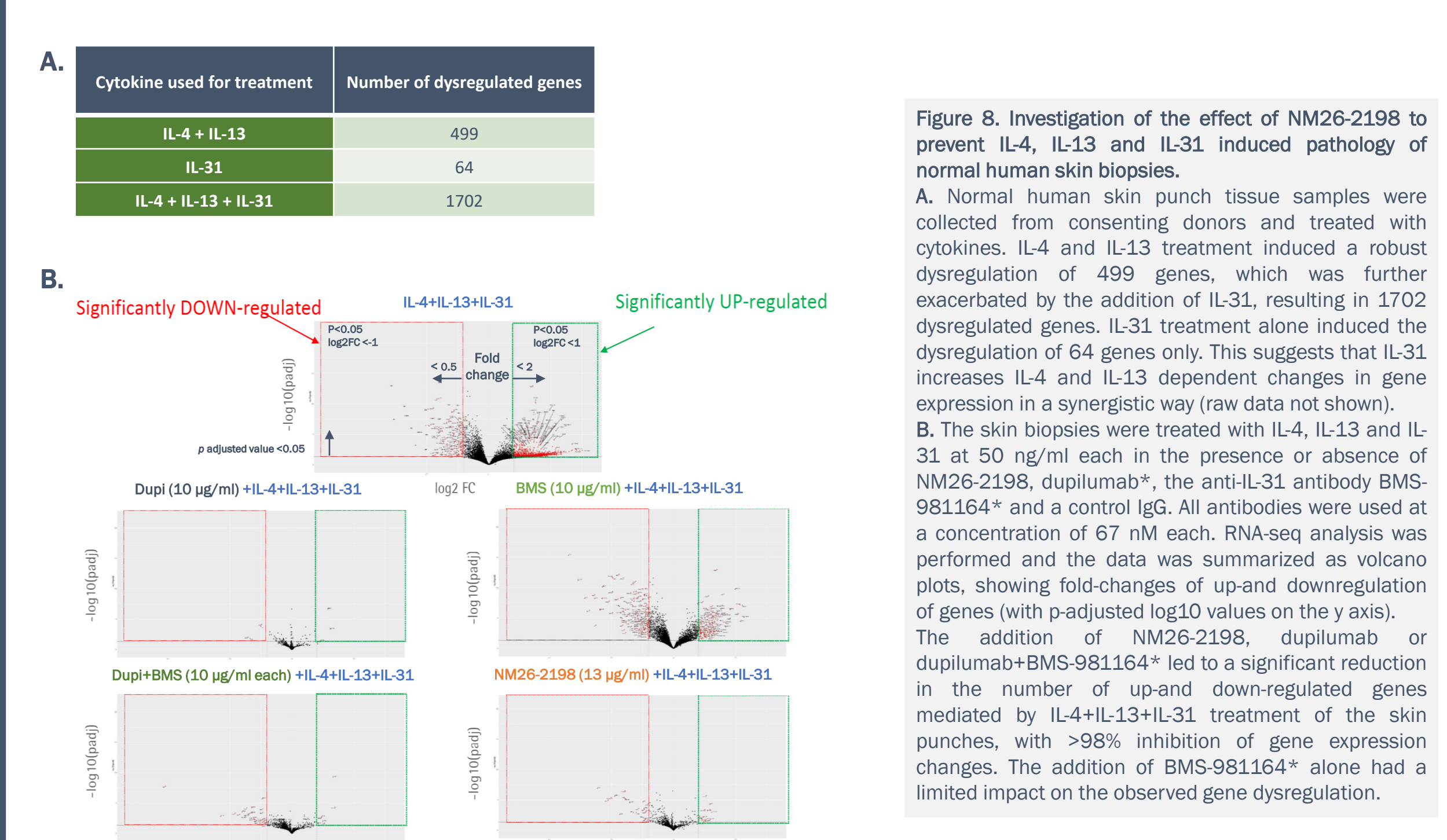


Figure 9. A. NM26-2198 binds to cynomolgus monkey IL-4R $\alpha$  with a high affinity of 123 pM as determined by multicycle kinetic SPR analysis using a T200 Biacore instrument. B. A KD of 120 pM was determined for NM26-2198 binding to cynomolgus IL-31 by single cycle SPR analysis. C. NM26-2198 also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

## NM26-2198 is cross-reactive with cynomolgus monkey IL-4R $\alpha$ and IL-31

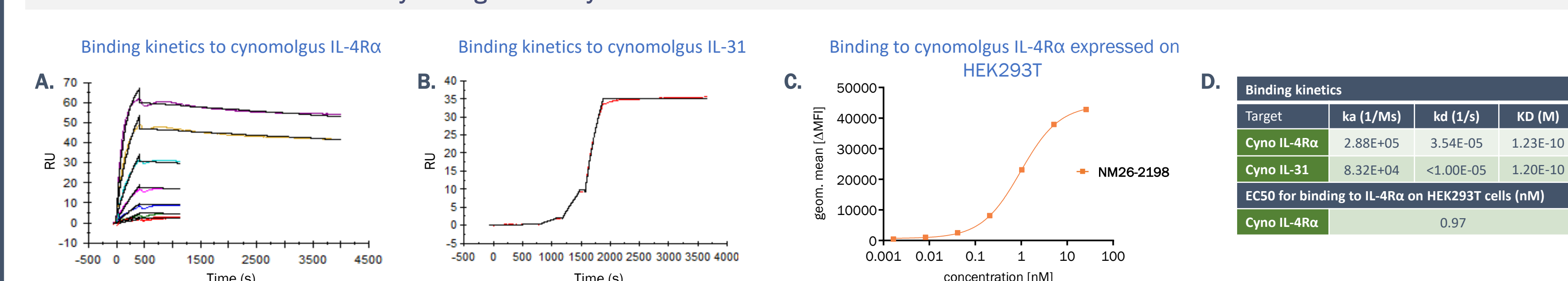


Figure 10. Effect of NM26-2198 on IL-31 induced pruritus in cynomolgus monkeys. A. Cynomolgus monkey IL-31 (cIL-31)-induced scratching was recorded in male monkeys on day -18 and the animals were randomized to treatment groups, excluding animals with strong baseline scratching behavior. On day 1, NM26-2198 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  $\mu$ g/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).

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

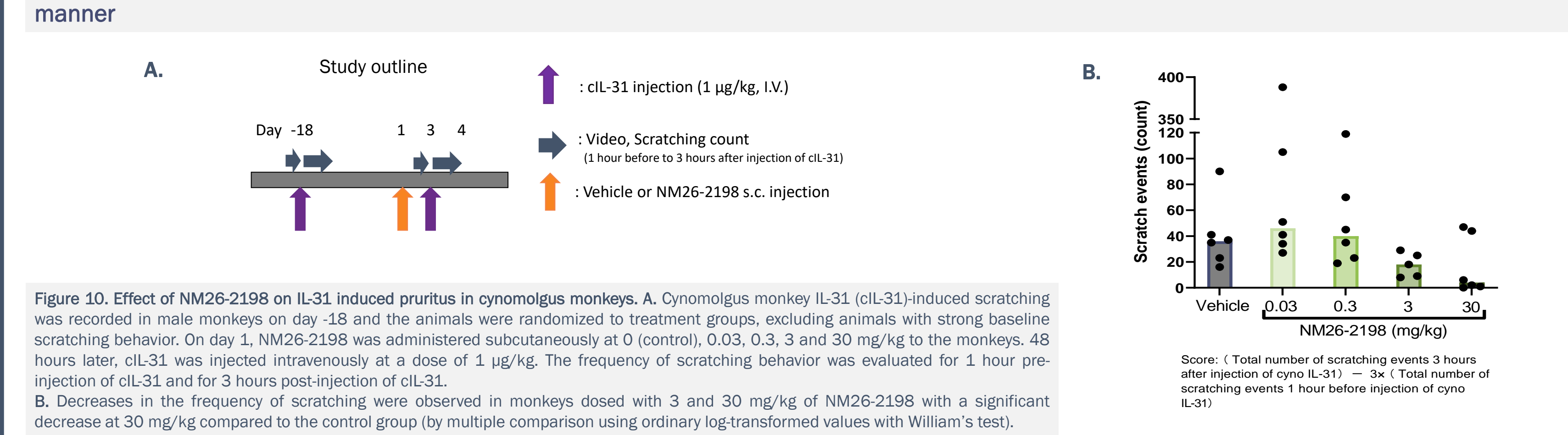


Figure 11. Pharmacokinetics in cynomolgus monkeys. A. NM26-2198 was administered i.v. to monkeys at 1, 5 and 25 mg/kg/day and s.c. at 5 mg/kg/day. B. NM26-2198 was injected s.c. at 25 and 125 mg/kg/day.

## 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<sub>1/2</sub> 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)