A call to arms

By Steve Edelson, Executive Editor

A paper by **Genmab A/S** researchers proposing a mechanism by which antibodies with IgG4 backbones may elicit side effects caught the attention of **PanGenetics B.V.**, which is running a Phase I psoriatic arthritis trial of PG102, an antibody with an IgG4 backbone that targets CD40. PanGenetics suspected the article might result in a flurry of questions from either investors or regulators on whether such backbones possess a class effect that could lead to safety issues.

PanGenetics put together data to support its contention that antibody backbones were actually a red herring and that a better place to look for the source of side effects would be a mAb's mechanism. Indeed, PanGenetics is not alone in developing antibodies with IgG4 backbones—there are at least 2 such antibodies on the market and 10 more in the clinic (see **Table 1, “IgG4 antibodies”**).

“Under scrutiny, the theory that IgG4 backbones can lead to side effects wilts.”

—Kevin Johnson,
PanGenetics B.V.

The IgG4 subclass of immunoglobulins is naturally anti-inflammatory and thus putatively a good starting point for developing therapies for diseases like psoriatic arthritis. A known drawback, however, is that IgG4 backbones can be unstable and thus short-lived in the body.

In July, the Genmab team reported in *Nature Biotechnology* that therapeutic antibodies with IgG4 backbones could swap Fab arms—the part of an antibody that binds antigens—with endogenous IgG4 antibodies *in vivo*.¹ That undesired exchange, the group wrote, could have “biological consequences.”

As a case study, the group showed that the marketed multiple sclerosis (MS) antibody Tysabri natalizumab from **Biogen Idec Inc.** and **Elan Corp. plc** underwent Fab-arm exchange with endogenous IgG4 antibodies.

According to the Genmab team, the Fab-arm exchange could potentially explain the rare cases of progressive multifocal leukoencephalopathy (PML) caused by Tysabri.² The paper was reviewed in *SciBX*.

“This paper has led to many questions relating to our own PG102 program,” said PanGenetics CEO Kevin Johnson. “PG102 is a native IgG4 and questions were raised whether patients weren’t being put at undue risk in the Phase I study of PG102.”

Johnson, who declined to disclose the sources of the questions, said PanGenetics “analyzed the available data for IgG4-based antibodies and

Table 1. IgG4 antibodies. There are a host of antibodies in development that have IgG4 backbones. Two have already reached the market and no fewer than five are in Phase II testing.

Company	Antibody	Target	Indication	Status
Biogen Idec Inc. (NASDAQ: BIIB)/ Elan Corp. plc (NYSE:ELN)	Tysabri natalizumab	Integrin α_4 (VLA-4; CD49D)	Multiple sclerosis (MS); Crohn's disease	Marketed
UCB Group (Euronext:UCB)/ Pfizer Inc. (NYSE:PFE)	Mylotarg gemtuzumab	CD33	Acute myelogenous leukemia (AML)	Marketed
TaiMed Biologics Inc.	Ibalizumab	CD4	HIV	Phase IIb
Altor BioScience Corp.	ALT-836	Tissue factor	Acute lung injury; acute respiratory distress syndrome	Phase IIb
AstraZeneca plc (LSE:AZN; NYSE:AZN)	CAT-354	IL-13	Asthma	Phase II
Facet Biotech Corp. (NASDAQ:FACT)/ Biogen Idec	Volociximab (M200)	Integrin $\alpha_5\beta_1$	Solid tumors	Phase II
Innate Pharma S.A. (Euronext:IPH)	Anti-KIR (IPH 2101; NN1975)	NK cells	Multiple myeloma	Phase II
Bristol-Myers Squibb Co. (NYSE:BMJ)	BMS-663513	CD137 (4-1BB)	Cancer	Phase I/II
Genzyme Corp. (NASDAQ:GENZ)	GC-1008	Transforming growth factor- β (TGFB; TGF β)	Malignant melanoma; renal cell carcinoma	Phase I/II
iCo Therapeutics Inc. (TSX-V:ICO)	iCo-008	Eotaxin-1 (CCL11)	Vernal keratoconjunctivitis	To start Phase II
Biotest AG (Xetra:BIO)	BT-062	Syndecan 1 (SDC1; CD138)	Multiple myeloma	Phase I
PanGenetics B.V.	PG102	CD40	Psoriatic arthritis	Phase I

concluded there wasn't a class effect. Under scrutiny, the theory that IgG4 backbones can lead to side effects wilts."

Johnson noted that Genmab's example included Tysabri and TGN1412, a CD28 agonist antibody with an IgG4 backbone from now insolvent TeGenero Immuno Therapeutics AG that caused serious inflammatory reactions in all six patients given the mAb in a first-in-man trial. "But these side effects are mechanism-of-action effects and not isotype effects," he said. "The TeGenero molecule was a superagonist that was given at a high dose and dramatically activated lymphocytes. That was not a consequence of its backbone."

Johnson noted that PML also occurs in patients given Rituxan rituximab, an anti-CD20 antibody from Biogen Idec and Roche's Genentech Inc. unit with an IgG1 backbone. Rituxan is marketed to treat non-Hodgkin's lymphoma (NHL) and rheumatoid arthritis (RA).

According to Johnson, a January paper from researchers at the Karolinska Institute provided a good argument against the Genmab team's hypothesized mechanism for what happens when Fab-arm exchange occurs between Tysabri and IgG4 antibodies against John Cunningham virus (JCV), an opportunistic infection that causes PML.

Tysabri targets integrin α_4 (VLA-4; CD49D) on white blood cells and prevents them from binding to vascular cell adhesion molecule-1 (VCAM-1), which is found on endothelial cells. The lymphocytes are unable to leave the vasculature and therefore are prevented from getting into the brain and attacking the myelin sheaths of neurons.

The Genmab group speculated in their paper that "circulating IgG4 antibodies against JCV might recombine with natalizumab by Fab-arm exchange. The JCV/VLA-4 bispecific antibodies generated could then mediate the capture of JCV by infiltrating activated leukocytes and thus facilitate the transport of JCV into the central nervous system."

The Karolinska team studied blood and cerebrospinal fluid from 217 MS patients and 212 controls and found low copy numbers of JCV DNA in 4 people—2 from each group.³ Thus, said Johnson, it is hard to see how the hybrid molecules proposed by the Genmab team "would encounter free virus to transport. Moreover, the postulated mechanism of that transport, infiltrating activated (VLA-4⁺) leukocytes, is precisely the mechanism that is inhibited by Tysabri."

Paul Parren, SVP of research and preclinical development at Genmab and corresponding author on the *Nature Biotechnology* paper, agreed with Johnson that there is "no proof of correlation with IgG4 backbones and

the side effects that have been seen. We don't want to alarm patients who are benefitting from treatments with IgG4 antibodies, and we even included a pharmacokinetics model in our paper that indicates that undesired cross-linking via Fab-arm exchange is quite unlikely to occur under normal conditions. We already tried to put potential risks into perspective."

Nevertheless, Parren thinks that antibodies with nonstabilized IgG4 backbones "should not be developed in the future. The reason is that combining of the drug with patients' IgG4 introduces unpredictability to its *in vivo* behavior. You can control for it now, so why take the risk."

Some companies stabilize the core-hinge region of their IgG4 backbones—thus preventing Fab-arm exchange—by introducing a point mutation. An example of this is Mylotarg gemtuzumab from UCB Group and Wyeth, which was recently acquired by Pfizer Inc. Mylotarg, a humanized antibody against CD33 that delivers the toxin calicheamicin, is approved to treat CD33⁺ acute myelogenous leukemia (AML) in patients with first relapse who are 60 or older and who are not considered candidates for other cytotoxic chemotherapy.

Mylotarg's point mutation blocks Fab-arm exchange, although the drug, like all others, still has side effects. Mylotarg's label warns of hypersensitivity reactions and hepatotoxicity.

In contrast, Genmab's approach is to remove the hinge region altogether with its Unibody technology, which produces half-sized antibody fragments.⁴

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COMPANIES AND INSTITUTIONS MENTIONED

Biogen Idec Inc. (NASDAQ:BIIB), Cambridge, Mass.
Elan Corp. plc (NYSE:ELN), Dublin, Ireland
Genentech Inc., South San Francisco, Calif.
Genmab A/S (CSE:GEN), Copenhagen, Denmark
Karolinska Institute, Stockholm, Sweden
PanGenetics B.V., Utrecht, the Netherlands
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
UCB Group (Euronext:UCB), Brussels, Belgium