



# ASN004, a Novel 5T4-Targeted Dolaflexin™ ADC for the Treatment of Various Cancers

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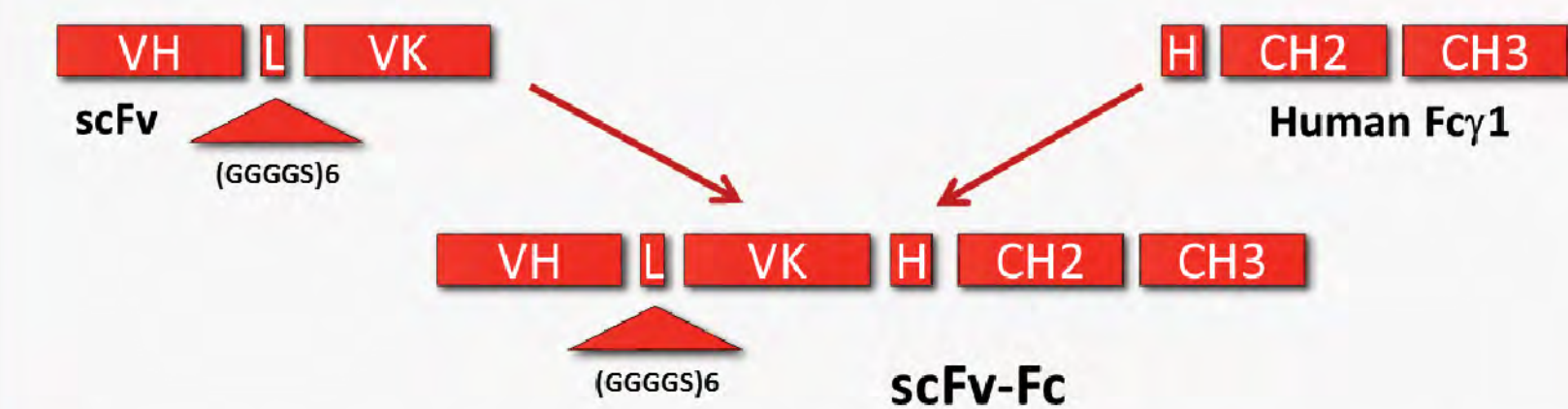
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## Abstract

ASN004 is an Antibody Drug Conjugate (ADC) that selectively targets tumor cells and employs Fleximer® linker technology (Mersana Therapeutics)<sup>3</sup>, for the treatment of a wide variety of cancers. ASN004 targets the 5T4 oncofetal antigen (trophoblast glycoprotein) that is highly expressed in a wide range of malignant tumors, while having very limited expression in normal tissues. ASN004 employs a novel scFvFc antibody for this antigen and delivers several cytotoxic dolastatin (auristatin) analog warheads per ADC molecule (drug/antibody ratio ~15). ASN004 shows potent, selective cytotoxicity in 5T4-expressing tumor cell lines, and provides complete and durable tumor regressions (90 days) in multiple tumor xenograft models at well-tolerated doses as low as <1 mg/kg iv. As well, a single iv dose of ASN004 was sufficient to afford complete and sustained regressions. Exploratory toxicology studies have been completed in both pharmacological and non-pharmacological non-clinical species. IND enabling safety studies with this promising new agent are planned to begin soon.

## 5T4 scFvFc Antibody

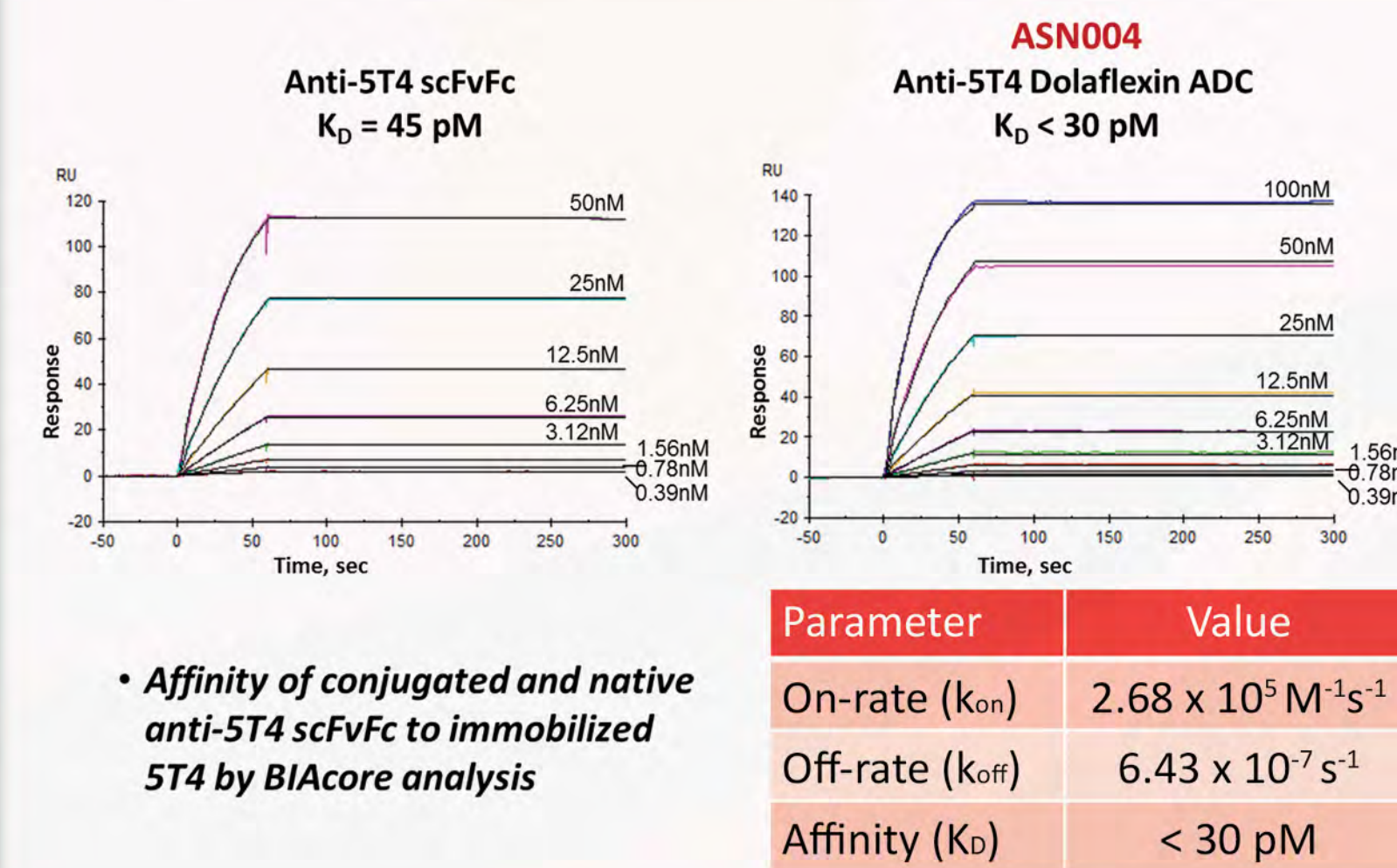
- Amino acid sequence of a Fab of a murine IgG1 anti-human 5T4 antibody was described by Forsberg and coworkers.<sup>6</sup>
- CDRs described for this murine Fab were grafted on the human VH3/VK3 framework and humanized scFvFc was assembled with the following orientation:



- Plasmid constructs expressing scFvFc fusion proteins were transfected in HEK293 cells for transient expression or CHO-DG44 cells for stable expression.
- An unpaired cysteine was introduced at the junction of VK and Hinge regions for optional site-specific chemical modifications.
- scFvFc protein secreted in the cell-free supernatant was purified using protein A affinity chromatography and characterized for biochemical and functional properties.
- The scFvFc protein is a disulfide-linked homodimer of ~110 kDa molecular mass.

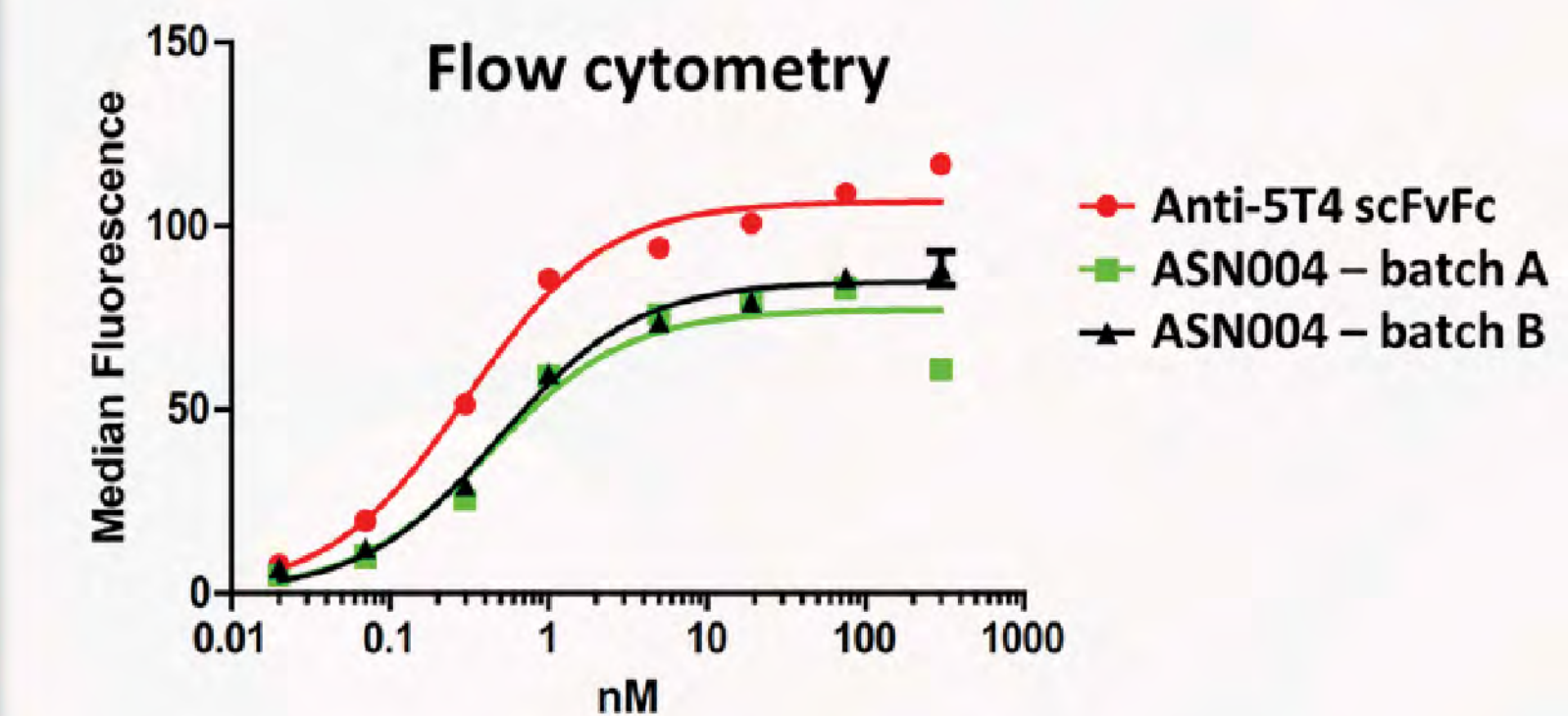
## ASN004 In Vitro Profile

High Affinity for the 5TA Antigen is Not Compromised Upon Formation of ASN004



High Affinity for A431 Epidermoid (5T4+) Tumor Cells is Not Compromised Upon Formation of ASN004

Parameter	Anti-5T4 antibody	ASN004 batch A	ASN004 batch B
A431 Binding EC <sub>50</sub> (nM)	0.31	0.49	0.43



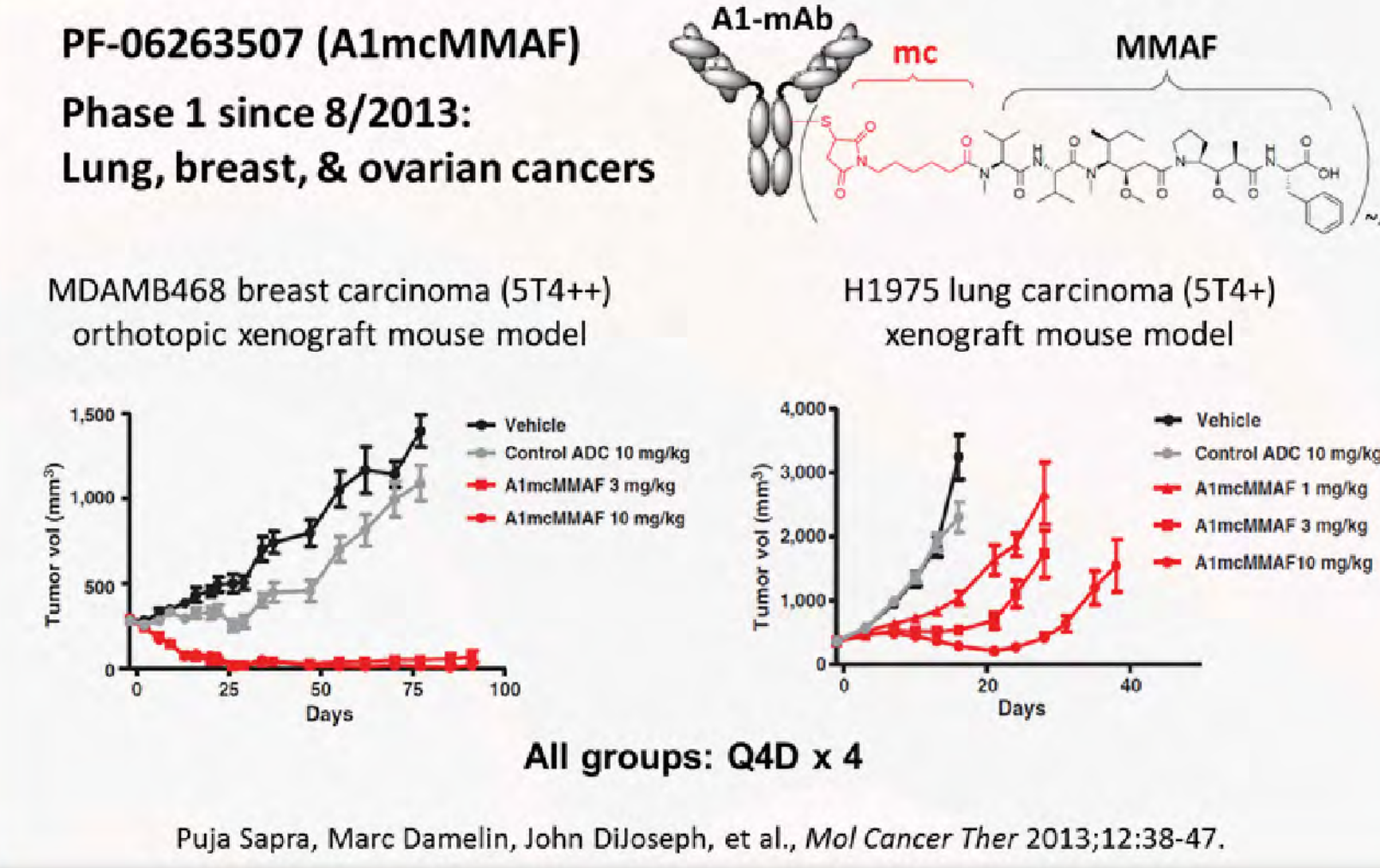
ASN004 Shows Potent and Selective Anti-Proliferative Activity in 5T4-Expressing Human Tumor Cell Lines

Test Article	Cell Proliferation IC <sub>50</sub> (nM)				
	A431 (5T4+)	PC3 (5T4+)	MDA-MB-231-5T4OE (5T4+++)	MDA-MB-231 (5T4-)	mouse LLC1
ASN004	0.3	0.6	< 0.1	NA	NA
anti-5T4 scFvFc	NA	NA	NA	NA	NA
warhead (AF-HPA)	0.9	2.5	--	--	NA

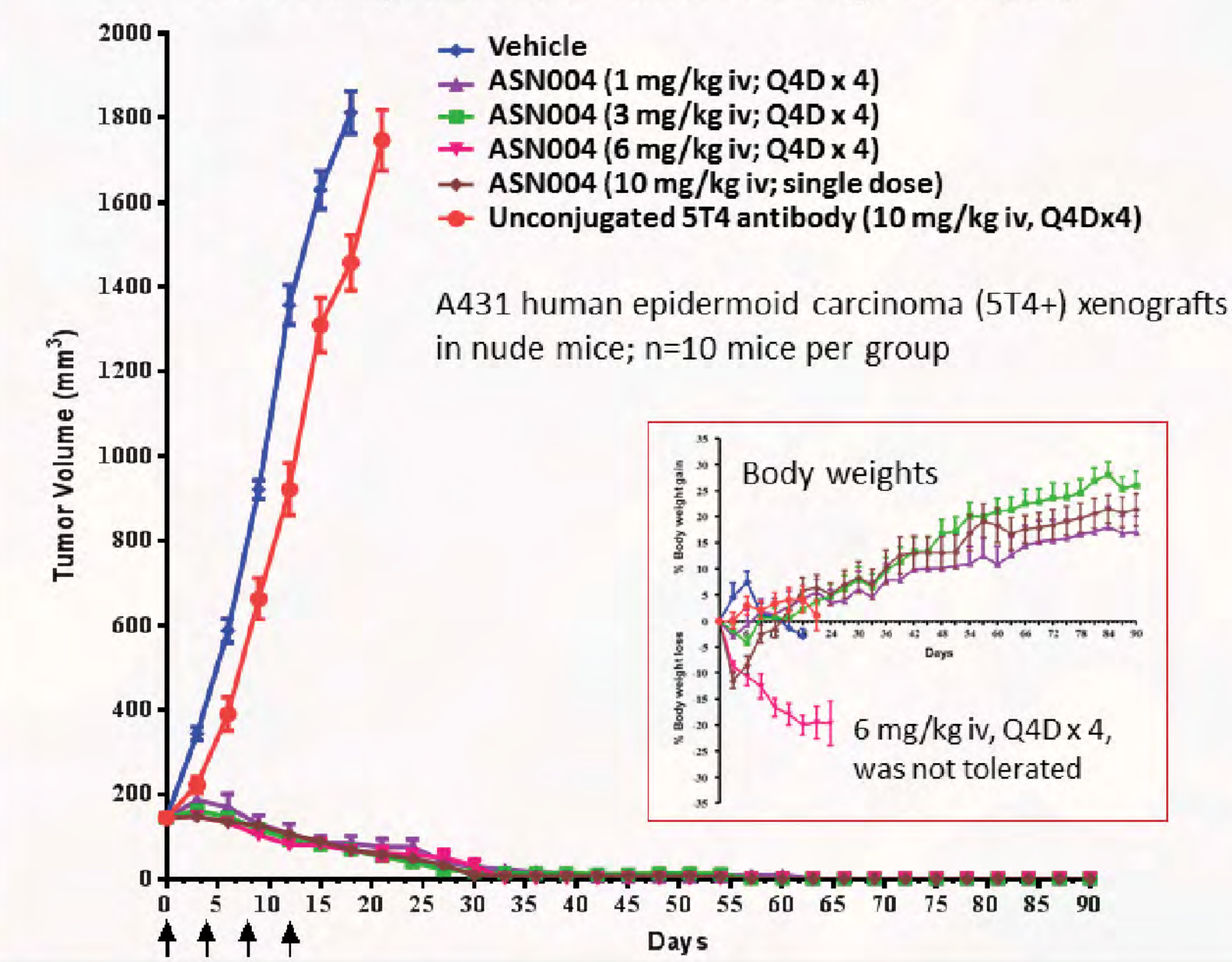
ASN004 is expressed in ADC equivalents; warhead is expressed in AF-HPA equivalents; NA: not active; --: not determined; A431: epidermoid carcinoma; PC3: prostate carcinoma; MDA-MB-231: breast carcinoma; MDA-MB-231-5T4OE: 5T4-over-expressing transfectant of MDA-MB-231; LLC1: mouse Lewis lung carcinoma

## ASN004 In Vivo Profile

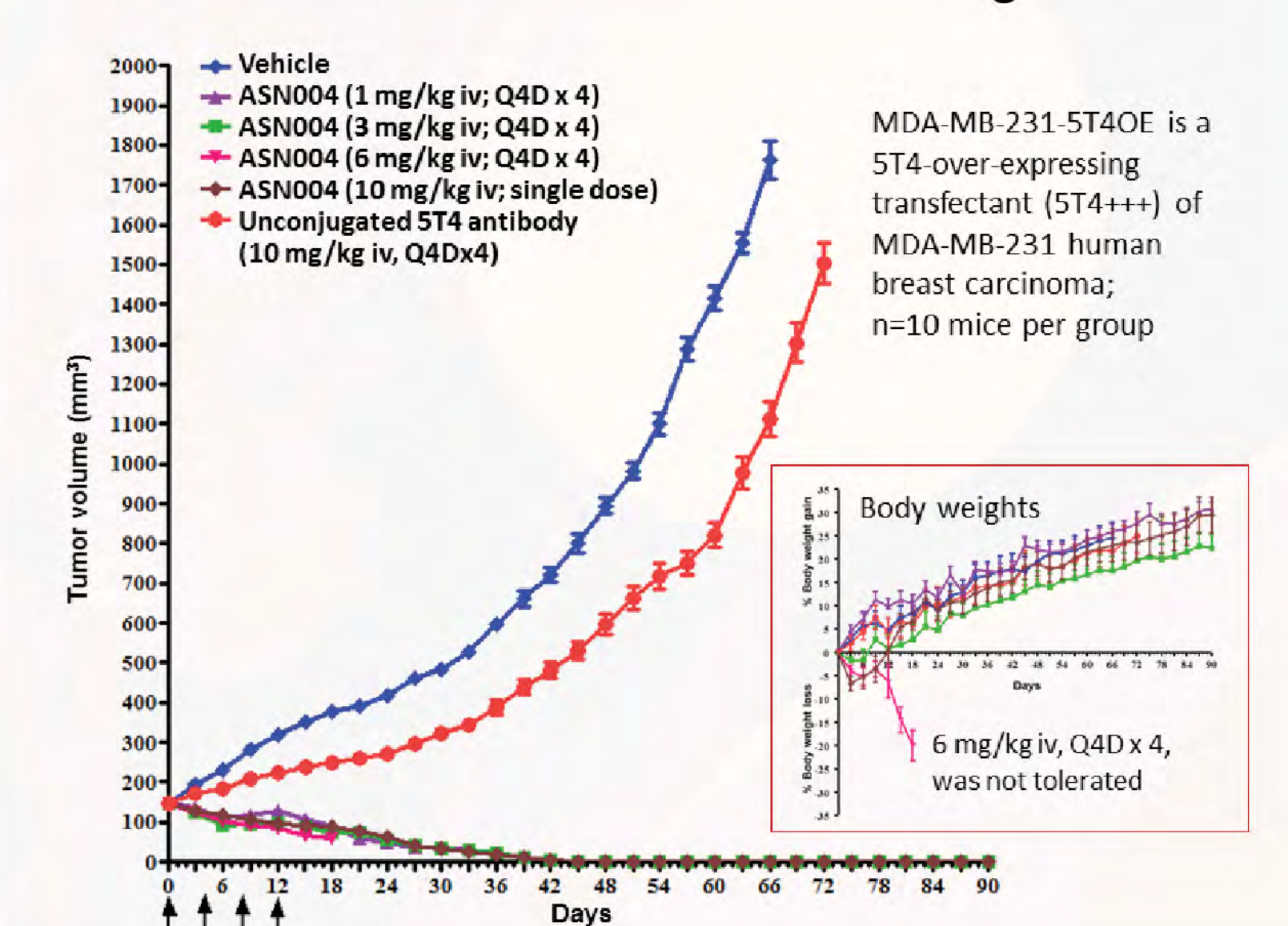
Reference ADC: Pfizer anti-5T4 ADC, PF-06263507



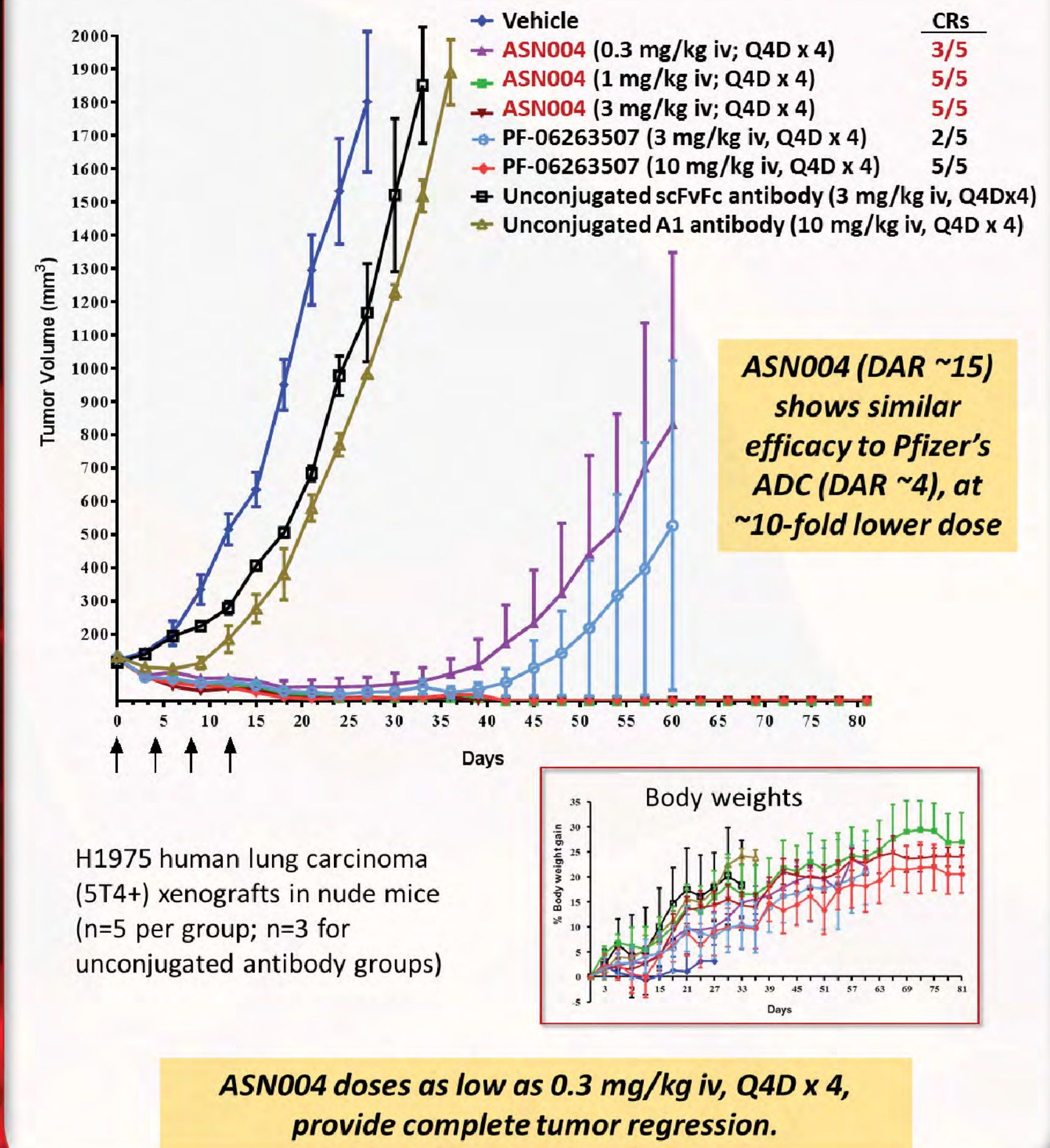
ASN004 Treatment Leads to Tumor-Free Survivors (90 days) in A431 Epidermoid Tumor Xenograft Model



ASN004 Treatment Leads to Tumor-Free Survivors (90 days) in MDA-MB-231-5T4OE Breast Tumor Xenograft Model

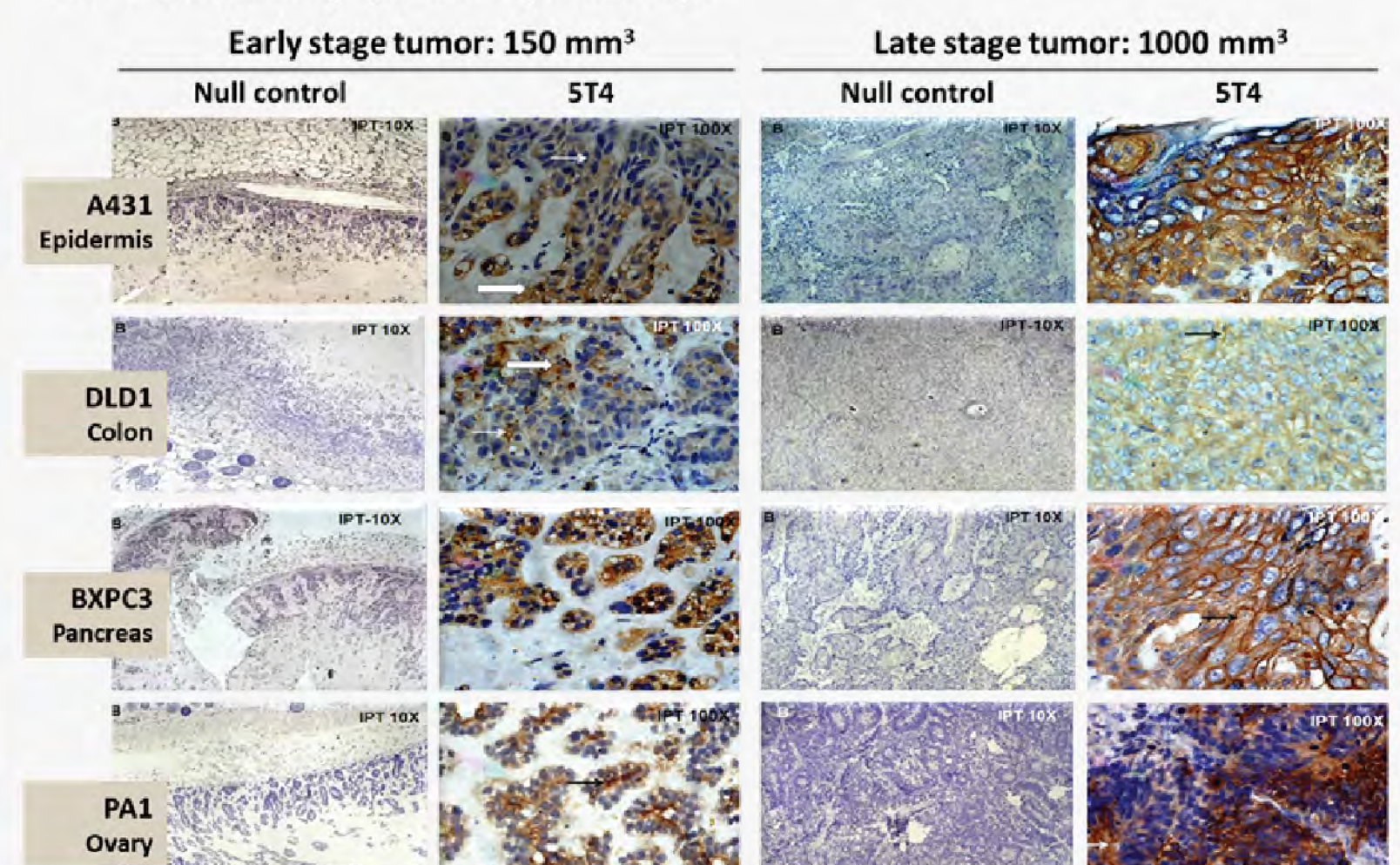


ASN004 Treatment Leads to Tumor-Free Survivors (81 days) in H1975 Lung Carcinoma Xenograft Model

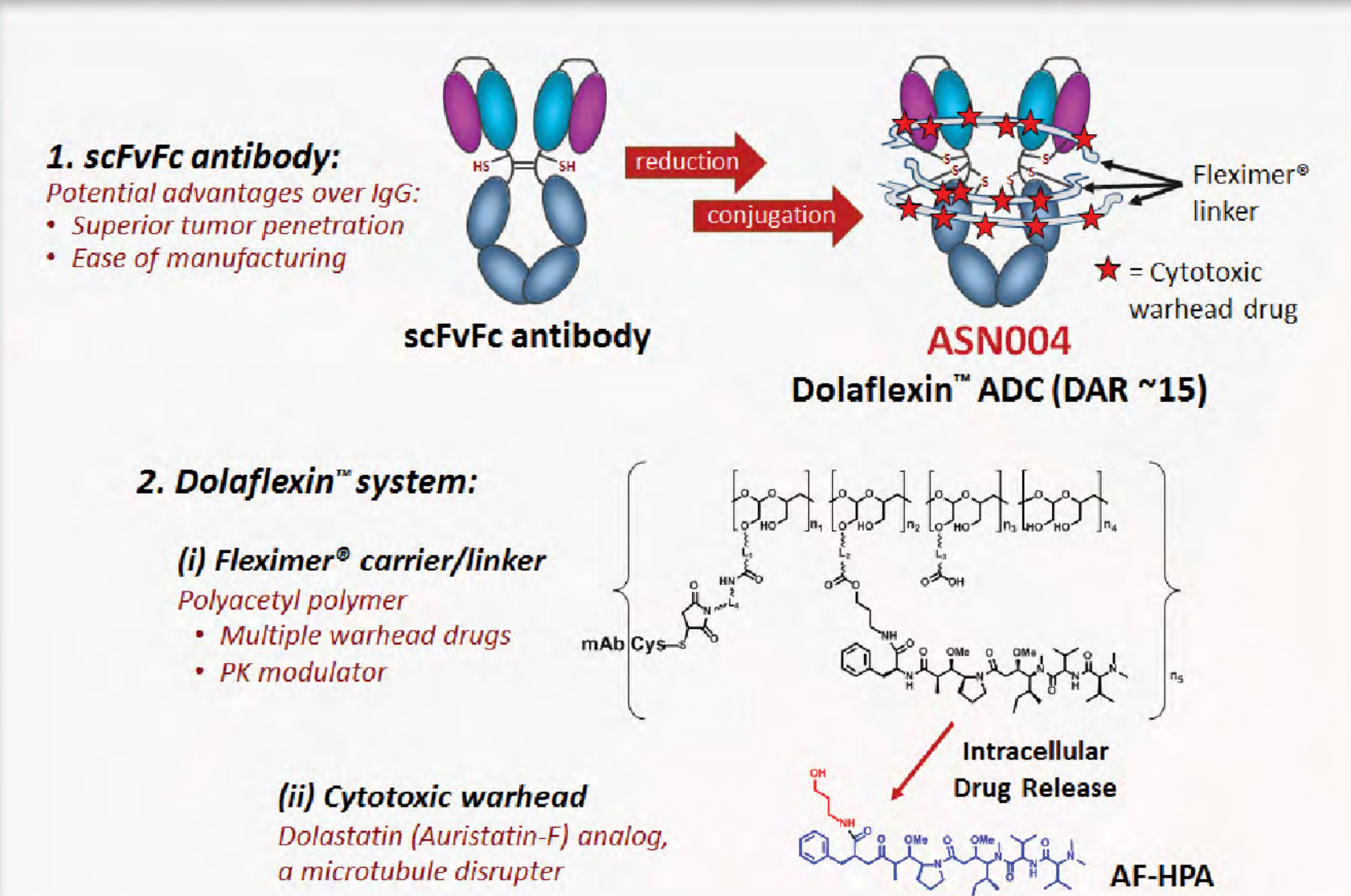


## 5T4 Oncofetal Antigen Target

- 72 kD heavily glycosylated transmembrane protein
- Widely expressed in malignant tumors throughout their development, but very limited expression in normal tissues<sup>2,3</sup>
- Expression on carcinomas and cancer stem cells correlates with metastatic tumor growth – prognostic marker
- mAb bound to 5T4 antigen is rapidly internalized within the cell – ideal for ADCs
- Antigen target for PF-06263507<sup>4,5</sup> (A1-mafodotin ADC, A1mCMMAF), Pfizer – currently in Phase I
- Immunohistochemical staining of 5T4 in mouse xenograft human tumor tissues, using sheep polyclonal anti-human 5T4 antibody (R&D Systems):



## ADC Synthesis and Structure



ADC Characterization		
Parameter	Analysis	Method
Drug/mAb ratio (DAR)	14.8	UV/LC-MS
Free mAb content, % total	<1%	HPLC
Free Drug content, % total	0.13%	LC-MS
ADC Apparent peak MW	179 kDa	SEC
Aggregated fraction content, % total	<5%	SEC

## ASN004 Summary

- Structural components:**
  - Antibody: anti-5T4, scFvFc structure
  - Linker: Fleximer® (3 Fleximer® chains per antibody)
  - Warhead: Dolastatin (Auristatin-F) analog; Drug:Antibody ~15
- High binding affinity (picomolar) to target antigen**
- Potent in vitro cytotoxicity**
- Potent in vivo anti-tumor activity in multiple human tumor xenograft models (e.g., epidermoid, breast, lung)**
  - Complete tumor regression observed with doses as low as 0.3 mg/kg iv, Q4D x 4
- Well-tolerated in exploratory toxicology studies**
  - Rat (non-pharmacological) and marmoset (pharmacological)
  - Observed toxicities are considered acceptable to support further development of ASN004
- IND-enabling GLP toxicology studies are being planned**

Acknowledgement: We wish to thank Priyaranjan Pattanaik and his colleagues at Syngene International Ltd for their work with the anti-5T4 antibody and on some characterization studies of ASN004. We also thank Sanjeeva Reddy (Asana BioSciences) for helpful discussions.

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