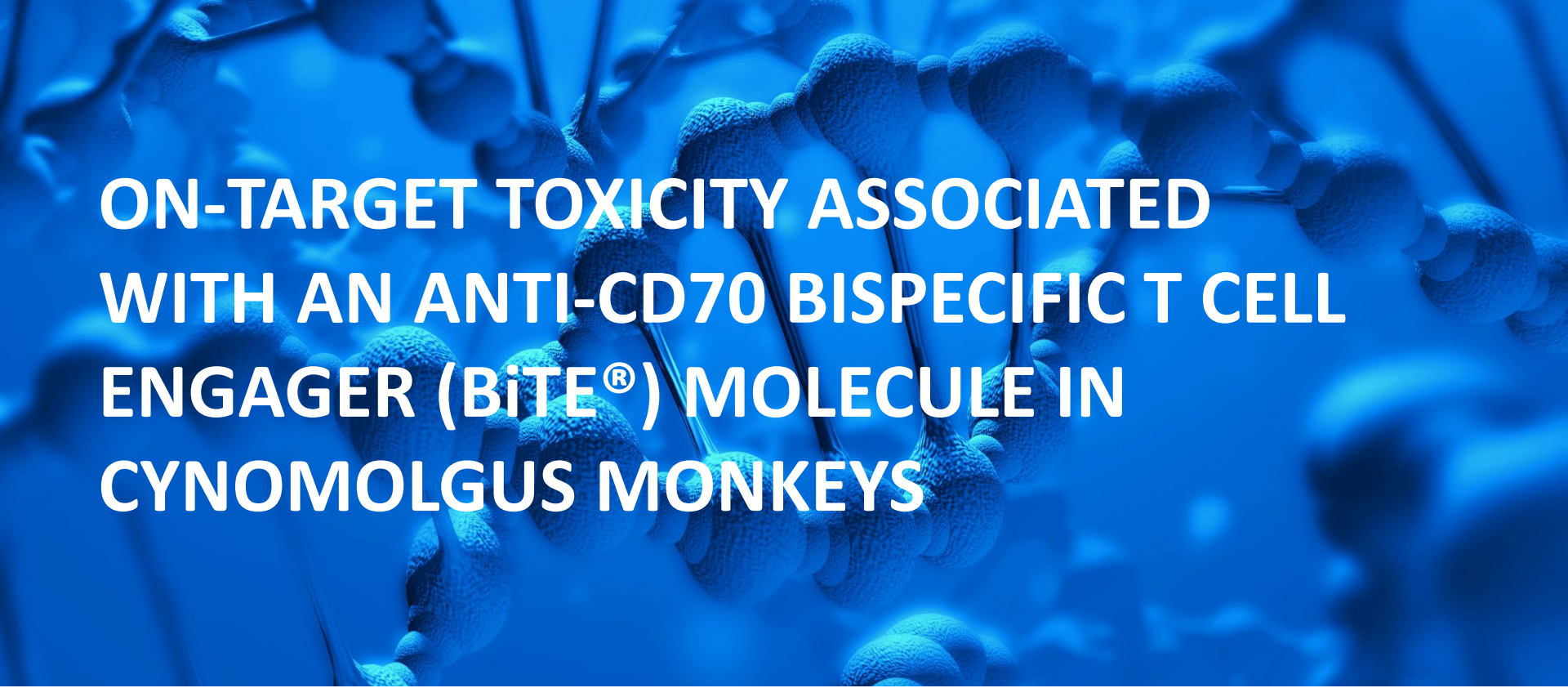

ON-TARGET TOXICITY ASSOCIATED WITH AN ANTI-CD70 BISPECIFIC T CELL ENGAGER (BiTE®) MOLECULE IN CYNOMOLGUS MONKEYS

TOD HARPER JR PHD, DABT

SEPTEMBER 14TH 2023 – SOT DDTSS WEBINAR

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  - Harper et al., Toxicological Sciences 2022 https://doi.org/10.1093/toxsci/kfac052

- **Amgen:** Jacintha Shenton, Graeme Moffat

- **All others who worked on this project over the years**

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PRESENTATION OVERVIEW

Background: Bispecific T cell Engager (BiTE®) Molecules and Nonclinical Safety Considerations

Case Study: Anti-CD70 BiTE Molecule N6P

Future Nonclinical Safety Assessments: Learnings & Recommendations

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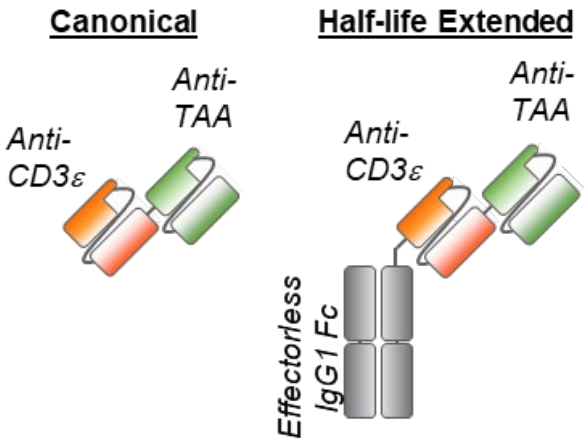
OVERVIEW OF BiTE® MOLECULES



Comparison of canonical BiTE and Half-life Extended (HLE) BiTE molecules

Bispecific T Cell Engager (BiTE) molecules:

- BiTE molecules are composed of two single-chain variable fragment domains (scFv) tethered to a human IgG1 Fc domain conferring half-life extended pharmacokinetic properties.

TAA = Tumor-associated Antigen

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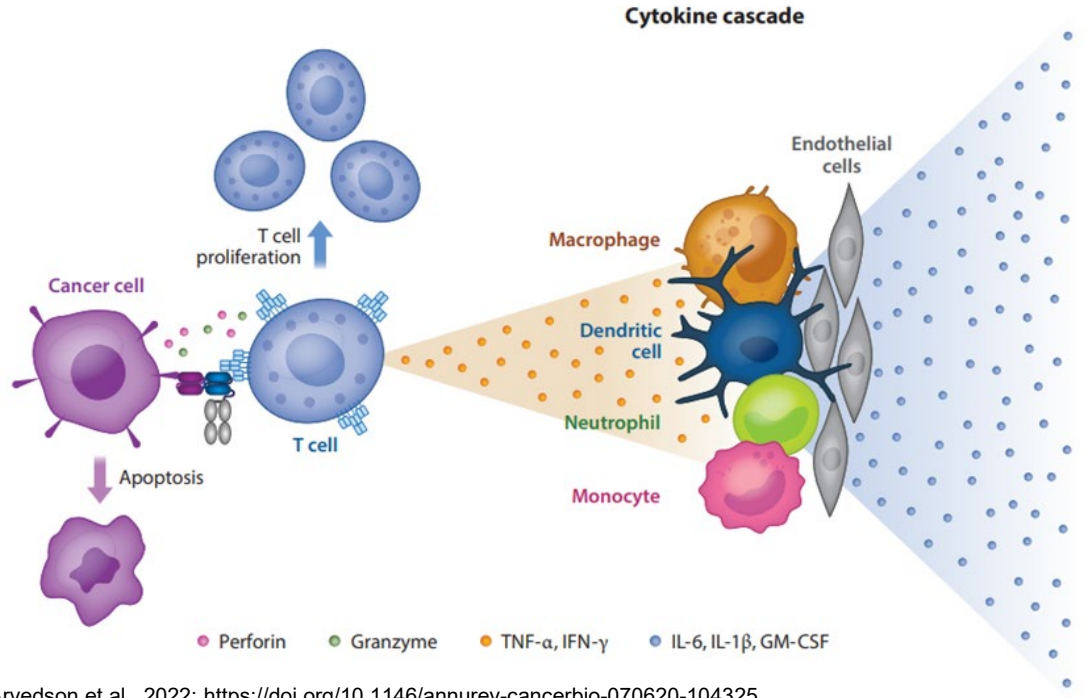
OVERVIEW OF BiTE® MOLECULES



Arvedson et al., 2022: https://doi.org/10.1146/annurev-cancerbio-070620-104325

<u>Mechanism of Action</u>:

Redirect T cells to cancer cells by binding to CD3 on T cells and a TAA on tumor cells.

T cell activation also causes **cytokine release** and **T cell proliferation**

Recognition of tumor cells **depends on the TAA and not on the major histocompatibility complex (MHC)**,

TAA = Tumor-associated Antigen
MHC = Major histocompatibility complex

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BiTE® MOLECULE NONCLINICAL SAFETY ASSESSMENT

ON-TARGET/ON-TUMOR

- The potential for on-target/on-tumor liabilities are often demonstrated during in vitro or in vivo antitumor activity studies as part of the pharmacological response

ON-TARGET/OFF-TUMOR

CD3-redirected cell lysis, inflammation, and associated events in non-tumor tissue expressing tumor TAA

Target Cell Killing Liability Assessment

Robust analysis of TAA expression level, tissue distribution, cellular (and subcellular) localization in normal tissues using literature, internal/external expression databases, and laboratory techniques e.g., flow cytometry and immunohistochemistry

In Vivo Nonclinical Safety Studies

The potential for on-target toxicities in non-tumor tissues which express the TAA are subsequently assessed during in vivo nonclinical safety studies in a relevant species

TAA = Tumor-associated antigen

Einsele et al. 2020 DOI: 10.1002/cncr.32909
Kamperschroer et al. 2020; DOI: 10.1080/1547691X.2020.1729902

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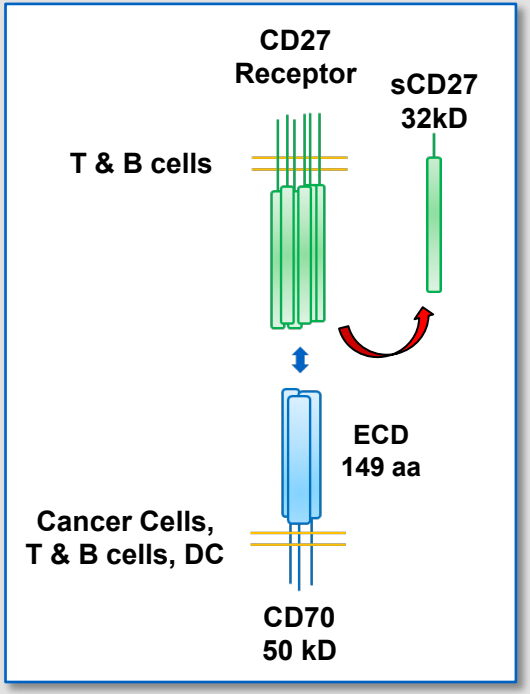
CASE STUDY: ANTI-CD70 BiTE® MOLECULE N6P

CD70: TARGET DESCRIPTION & RATIONALE

- **Type II transmembrane glycoprotein** forming homo-trimers
- **Member of the TNF receptor ligand family**
- **Cognate ligand** for the CD27 receptor
- **No soluble CD70** reported in human plasma but soluble CD27 is shed by activated T cells (Max serum level ~ 2,500 pg/ml) [1, 3]
- **Normal expression** in activated B and T cells, dendritic cells [2]
  - Subtle functions in T cell co-stimulation
- **Aberrantly expressed on tumors** such as renal cell carcinoma, leukemia, non-small cell lung cancer among others
- Numerous industry efforts to **target CD70 with varying mechanisms** (antibodies, chimeric antigen receptor, antibody drug conjugate)



[1] Huang J et al (2013) J Immunol 190 (12):6250-8
[2] Tesselaar K et al (2002) J Immunol 169: 33-40
[3] Ruf M et al. (2015) Clin Canc Res 21 (4) 889-98

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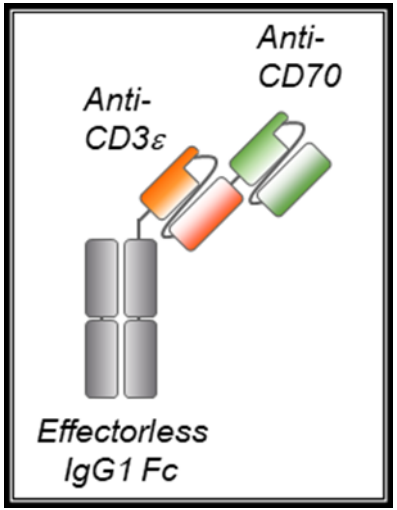
OVERVIEW OF CD70 BiTE® MOLECULE N6P



Anti-CD70 BiTE® Molecule N6P:

- Equipotent in human and NHP in vitro assays
- Specific (binding and killing)
- Potent (200x more potent than CD70 mAb format)
- Kills target cells with a much lower EC_{50} than activated T cells
- Not affected by soluble CD27

Harper et al., 2022 https://doi.org/10.1093/toxsci/kfac052

CD70 TARGET CELL KILLING LIABILITY ASSESSMENT

mRNA And Protein Levels Are Restricted In Normal Tissue

- CD70 expression is minimal and restricted to B and T lymphocytes
- In normal tissues, occasional lymphocytes were the only cells to stain CD70 positive in cynomolgus monkey and human and attributed to lymphocyte infiltration
- Expression profiles comparable between human and cynomolgus monkey tissues

In Some Immune Cell Types, CD70 Is Upregulated After Activation

- CD70 expression in T cells is greatly increased after *in vitro* stimulation/activation protocols
- Literature reports indicate inducibility in other cell types

Potential For CD70 Upregulation And Modulation In Other Cells And Tissues Is Not Well Understood

- Upregulation of CD70 in inflammatory conditions (as reported in literature)
- Degree (duration, extent) of upregulation is not known

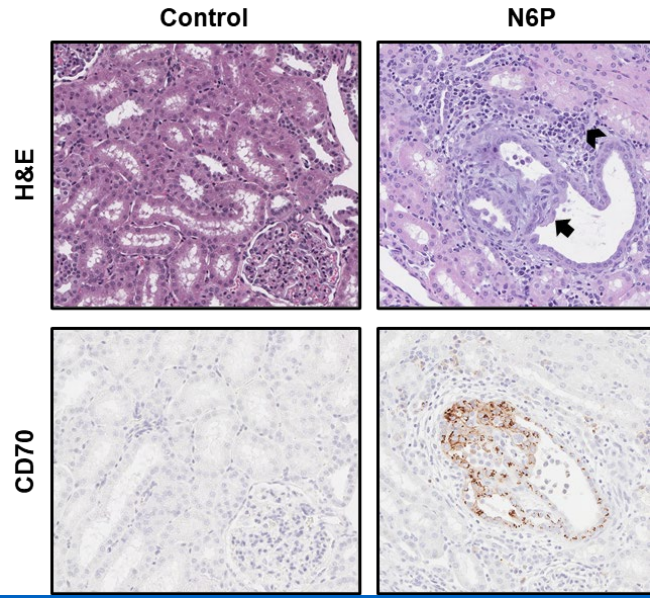
IN VIVO NONCLINICAL PK AND TOXICOLOGY STUDIES

Exploratory single dose pharmacokinetics study in cynomolgus monkeys

Exploratory repeat dose toxicology study in cynomolgus monkeys

GLP 1-month repeat dose toxicology study in cynomolgus monkeys

SINGLE DOSE NHP PK STUDY IDENTIFIED FINDINGS OF POTENTIAL CONCERN IN KIDNEY AND HEART



Degeneration/regeneration in proximal tubule w/ correlating CD70 expression in epithelium

Major Study Findings:

- Delayed general lethargic state with tremors; inflammatory cell infiltrates and/or inflammation with degeneration/necrosis of heart myofiber and renal tubules (single dose IV, necropsy at day 19)
  - **Similar findings** as in a prior canonical BiTE step dosed continuous IV; necropsy day 16

Investigational IHC:

- Expression of CD70 in regenerative renal tubule epithelium, correlating with sites of damage
  - Literature suggests CD70 expression can be upregulated in inflammatory settings in humans (lymphocytes, gut epithelia)
  - CD70 heart expression was identified in rare lymphocytes, but not in cardiac myofibers

Harper et al., 2022 https://doi.org/10.1093/toxsci/kfac052

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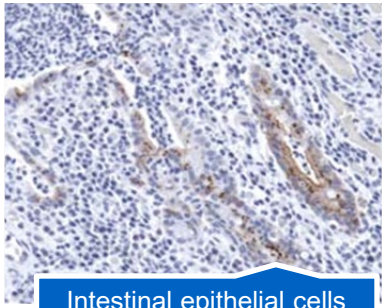
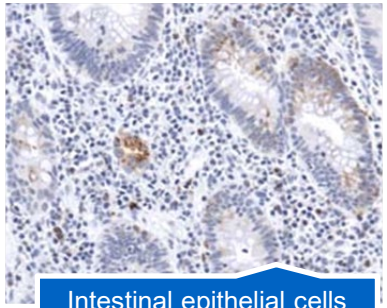
CD70 IS EXPRESSED IN EPITHELIUM UNDER VARIOUS INJURIES OR PROLIFERATIVE STATES

Chronic Inflammatory Disease

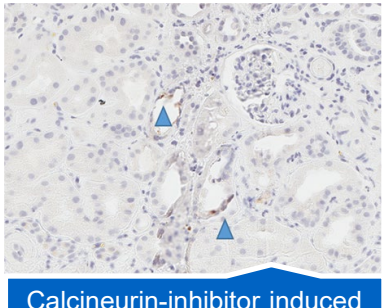


Intestinal epithelial cells from Crohn's disease patient samples

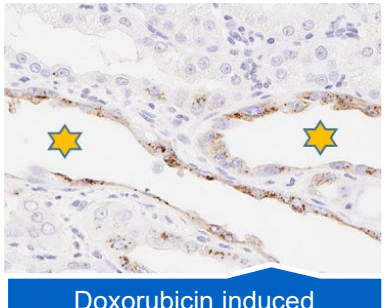


Intestinal epithelial cells from ulcerative colitis patient samples

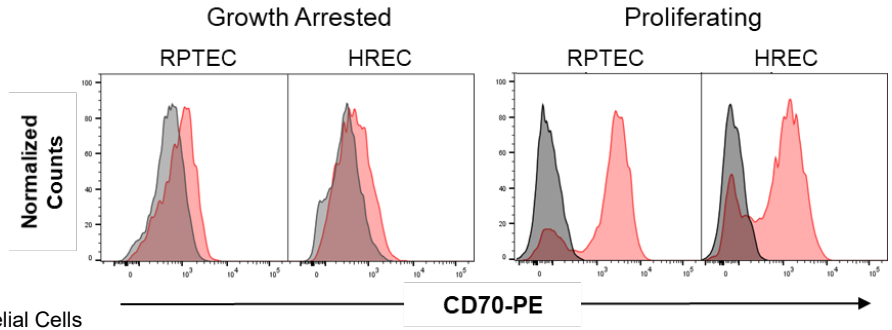
Drug-Induced Kidney Injury



Calcineurin-inhibitor induced degenerative/regenerative epithelium



Doxorubicin induced degenerative/regenerative tubular epithelium



RPTEC = Renal Proximal Tubular Epithelial Cells
HREC = Human Renal Cortical Epithelial Cells

Harper et al., 2022 https://doi.org/10.1093/toxsci/kfac052

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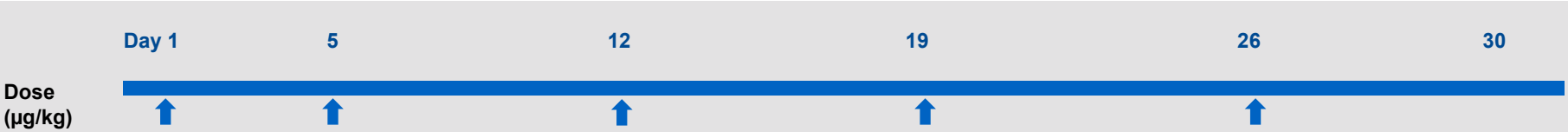
IN VIVO NONCLINICAL PK AND TOXICOLOGY STUDIES

Exploratory single dose pharmacokinetics study in cynomolgus monkeys

Exploratory repeat dose toxicology study in cynomolgus monkeys

GLP 1-month repeat dose toxicology study in cynomolgus monkeys

GLP 1-MONTH TOX STUDY DESIGN



Group 1	Vehicle control (VC)	VC	VC	VC	VC	Scheduled necropsy Begin Recovery
Group 2	2 ug/kg	2	2	2	2	Scheduled necropsy
Group 3	8 ug/kg	8	8	8	8	Scheduled necropsy
Group 4	2 ug/kg	8	24	24	24	Scheduled necropsy Begin Recovery

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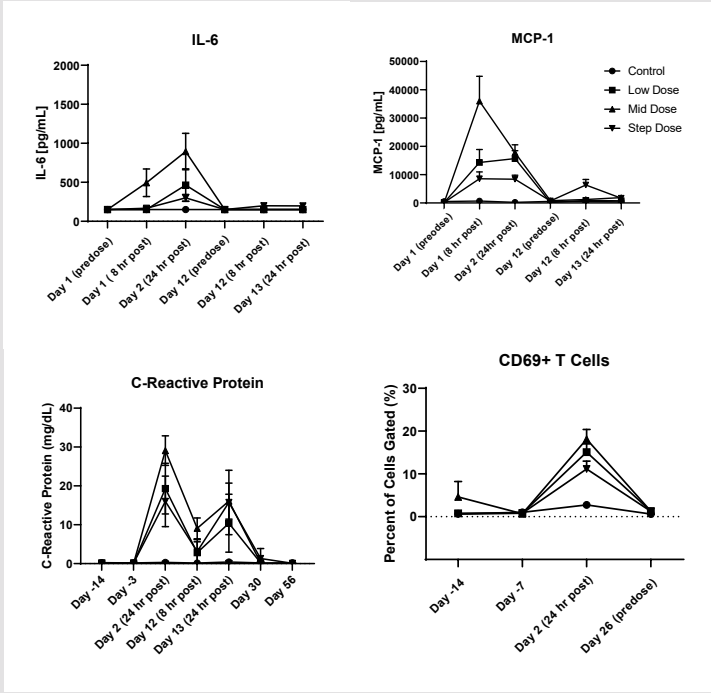
GLP 1-MONTH TOXICOLOGY STUDY SUMMARY

- **No unscheduled necropsies**
- **Clinical Observations:**
  - ≥ 2 ug/kg hunched posture, decreased activity/weakness, decreases in body weight in some animals ranging between 0.3 and 0.5 kg
    - Some animals recovered BW by day 30 and all animals fully recovered BW by day 57
  - ≥ 8 ug/kg generalized redness, dry skin, tremors
- **All dosed animals developed anti-drug antibodies (ADA) by end of study**
  - Development of ADAs was associated with decreased exposure starting at Day 12 (dose 3)
- **No changes in ophthalmic examinations**
- **Safety Pharm Endpoints:**
  - No changes in neurological examinations, body temperature, and respiration rates
  - ≥ 2 ug/kg increase in heart rate with an associated shortening of the RR interval, PR interval, and raw QT interval
    - All animals recovered or trended toward recovery by day 28 and completely recovered by day 57
    - All ECGs considered qualitatively normal
- **Hematology, clinical chemistry, and coagulation parameters consistent with an acute phase response within 24h of the first dose (2 or 8 µg/kg)**
- **Hallmarks of BiTE molecule activity**
  - Transient increase in cytokines, inflammatory response, decreased lymphocytes, monocytes, T, B cells, and T-cell activation



Harper et al., 2022 https://doi.org/10.1093/toxsci/kfac052

GLP TOXICOLOGY STUDY TARGET ORGANS AT ALL DOSE LEVELS

Macroscopic Observations:

- Liver (adhesions; pale white focus) at ≥ 2 µg/kg and in the heart (white focus on the epicardium), lung (adhesions), and lymph nodes (enlarged) in step-dose group

Histopathological Findings:

- **HEART:**

  Minimal to moderate chronic mixed cell inflammation of pericardium/epicardium (18 out of 18)

- **LIVER:**

  Minimal to moderate capsular fibrosis often with inflammation and macroscopic adhesions (13 out of 18)

- **LUNG:**

  Minimal to moderate pleural inflammation and fibrosis with macroscopic adhesions (7 out of 18)

- **KIDNEY:**

  Minimal to moderate mixed cell inflammation and tubular degeneration/regeneration (16 out of 18)

Theses histopathological changes are considered adverse with no clear dose-response

Harper et al., 2022 https://doi.org/10.1093/toxsci/kfac052

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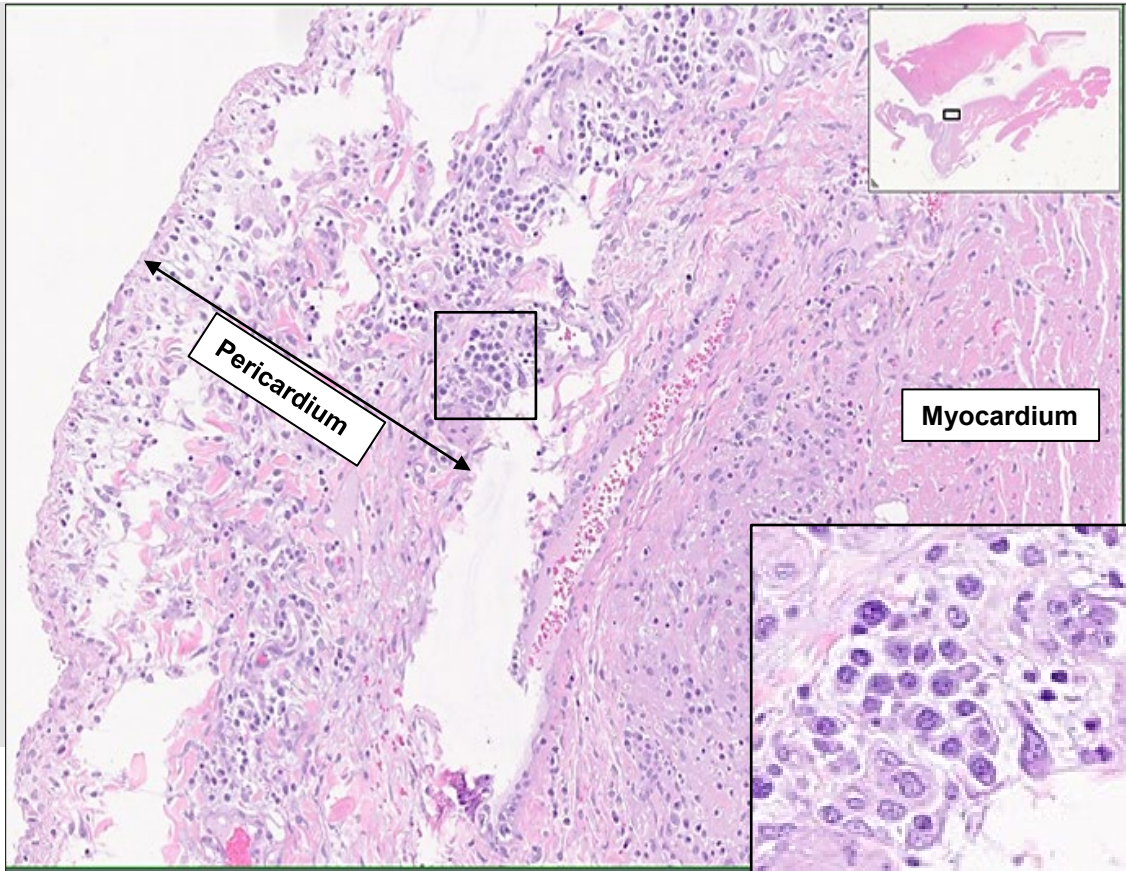
REVIEW OF KEY MICROSCOPIC FINDINGS

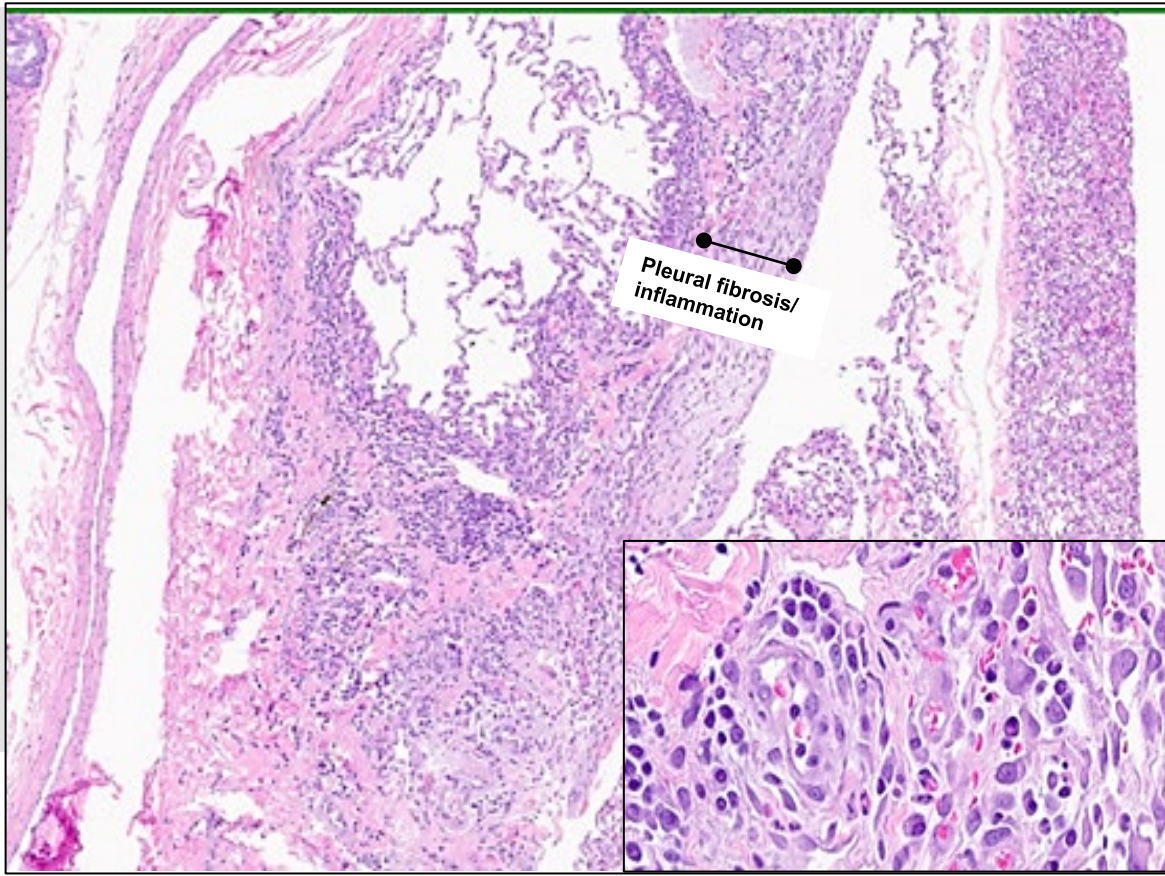
Heart, Pericardium: Inflammation, Mixed Cell

- All dose levels; minimal to moderate; lacked a clear dose-response relationship
- Characterized by chronic inflammation with thickening (arrow) of the pericardium, edema, and degeneration of mesenchymal cells
- Correlated with pale linear streaks on the epicardial surface of the ventricles at necropsy

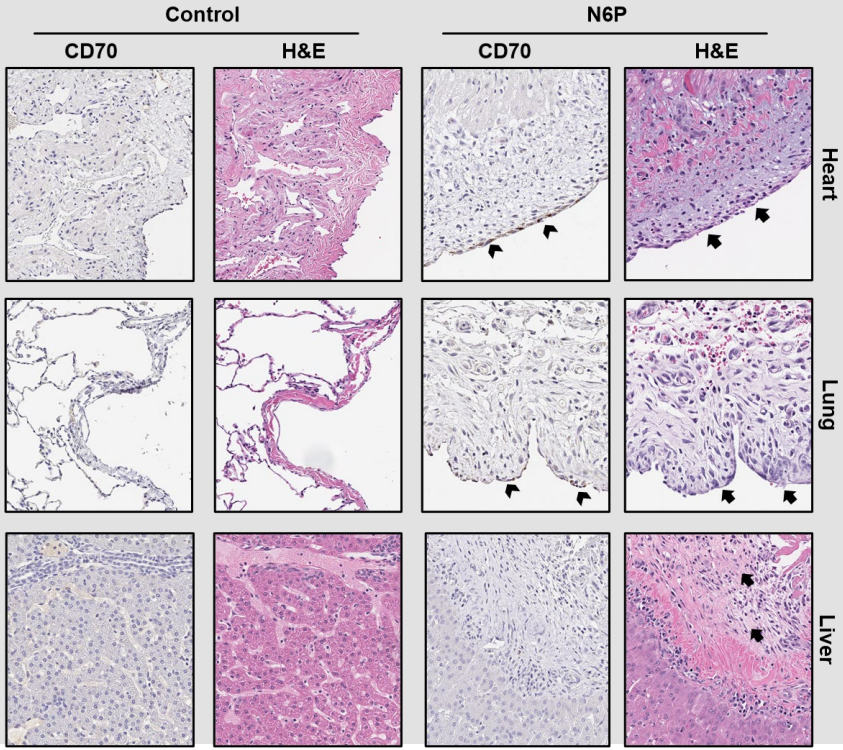


Lung, Pleura: Inflammation with Fibrosis

- Characterized by a mixed inflammatory cell infiltrate, thickening of the pleura, edema, cellular degeneration, and hyperplasia of adjacent type II pneumocytes
- Correlated with pleural adhesions



INDUCTION OF CD70 AT SITE OF INJURY



Positive CD70 staining associated with reactive pericardial mesothelial cells in the heart, and reactive pleural mesothelial cells of the lung from an N6P treated cynomolgus monkey.

No CD70 staining was observed in liver lesions, nor in control treated animals.

Harper et al., 2022 https://doi.org/10.1093/toxsci/kfac052

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ANTI-CD70 BITE MOLECULE: NONCLINICAL SAFETY SUMMARY

- **Multiple exploratory studies were conducted with N6P and other CD70 BiTE molecules to identify liabilities**
- **Key target organs identified during exploratory studies included kidney, heart, liver, and lung**

- **CD70 canonical BiTE Study - cIV**
- **N6P and 2 other CD70 HLE BiTE molecules - Single dose**
- **N6P 28-day repeat dose study**

- **The GLP toxicology study confirms target organs and reinforces concern of N6P-mediated toxicity at anticipated minimum efficacious exposure**

- **HEART**: Minimal to moderate chronic mixed cell inflammation of pericardium/epicardium
- **LIVER**: Minimal to moderate capsular fibrosis often with inflammation and macroscopic adhesions
- **LUNG**: Minimal to moderate pleural inflammation and fibrosis with macroscopic adhesions
- **KIDNEY**: Minimal to moderate mixed cell inflammation and tubular degeneration/regeneration

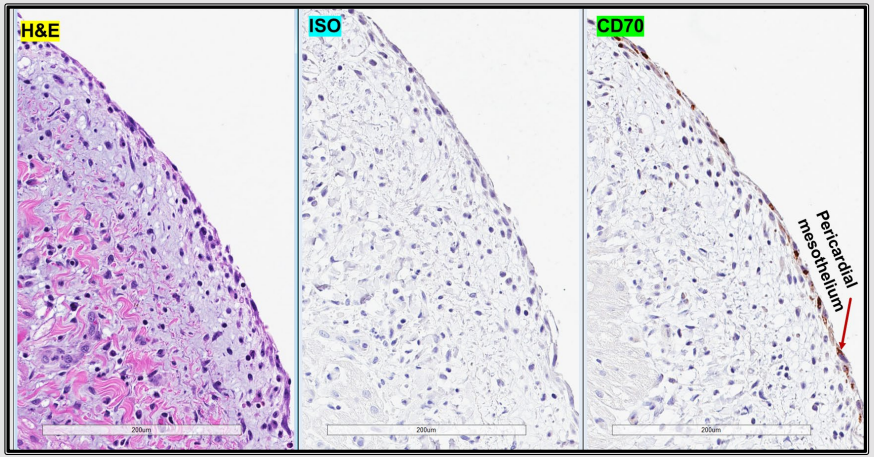
➢ **Upregulation of CD70 on mesothelium and tubule epithelium**

Serious, irreversible, and difficult to monitor findings in heart, liver, lung, and kidney

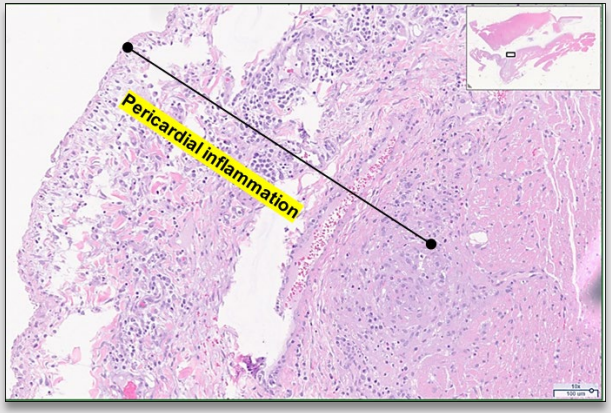
LEARNINGS AND RECOMMENDATIONS

LEARNINGS: CD70 EXPRESSION IS DYNAMIC AND CAN BE INDUCED DURING TIMES OF STRESS

Cancer	• Renal Cell Carcinoma, Acute Myeloid Leukemia, Mesothelioma
Inflammatory disease	• Intestinal epithelial cells from Crohn's disease and ulcerative colitis patient samples
Drug induced injury	• Doxorubicin induced degenerative/regenerative tubular epithelium • Calcineurin-inhibitor induced degenerative/regenerative tubular epithelium
CD70 BiTE molecule studies	• Kidney proximal tubule epithelium undergoing degeneration/regeneration • Mesothelial lining associated with inflammatory damage



CONCLUSIONS & RECOMMENDATIONS



- For some target antigens such as CD70, BiTE molecules may exhibit activity in tissues with very low antigen expression or the antigen maybe upregulated under stress enabling molecule activity.
- This work illustrates how a thorough understanding of expression and upregulation is needed to fully address putative liabilities associated with on-target/off-tumor activity of CD3 bispecific molecules.
- Comorbidities are typical in cancer patients, the due diligence lies with the Sponsor to understand the full extent of potential toxicity.
- Based on the CD70 experience, if there is any biological indication a target may be modulated in various states including diseases, we believe a robust drug development package should include investigation into such databases or assays, where possible.

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THANK YOU!