Mechanism of cysteine-mcMMAF release from the antibody-drug conjugate SGN-75

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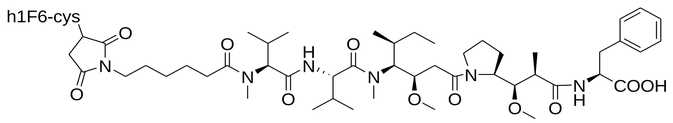
Abstract No. 5334

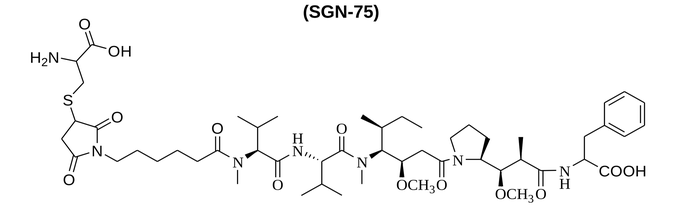
1. Abstract

The antibody-drug conjugate SGN-75 is composed of the humanized monoclonal antibody (mAb) h1F6 and the drug-linker mcMMAF conjugated through interchain disulfide cysteine residues. SGN-75 has been shown to be highly active against tumor cell lines both *in vitro* and *in vivo*. The mcMMAF drug-linker was designed to be protected from protease cleavage, and we have shown that cysteine-mcMMAF is the major released drug *in vitro*. We demonstrate that the cysteine residue in cysteine-mcMMAF comes from the mAb, implying antibody degradation as the mechanism for generation of cysteine-mcMMAF. Two isotope cysteine labeling approaches were utilized in which either total cysteine in tumor cells or the mAb cysteines of h1F6 were labeled with heavy isotope cysteine. Metabolic incorporation of heavy cysteine into CD70-expressing tumor cells or mAb-producing CHO cells using media depleted of cysteine and supplemented with heavy isotope cysteine gave complete labeling by heavy cysteine. Heavy cysteine-labeled cells were treated with unlabeled SGN-75, and unlabeled cells were treated with heavy-cysteine-labeled SGN-75. The results demonstrate that the mAb backbone of SGN-75 is the origin of the cysteine in cysteine-mcMMAF, providing evidence that an active drug component can be released from a stably linked ADC through proteolytic degradation of the mAb inside of target cells.

2. Structure of Antibody- Drug Conjugate



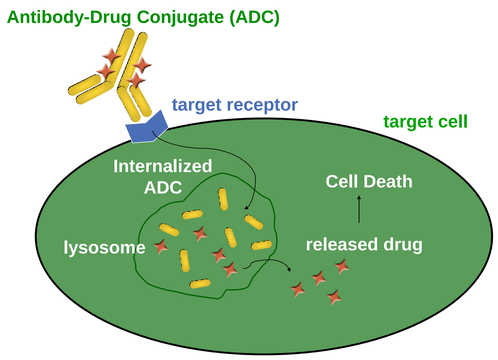
h1F6-mcMMAF
(SGN-75)



Cys-mcMMAF

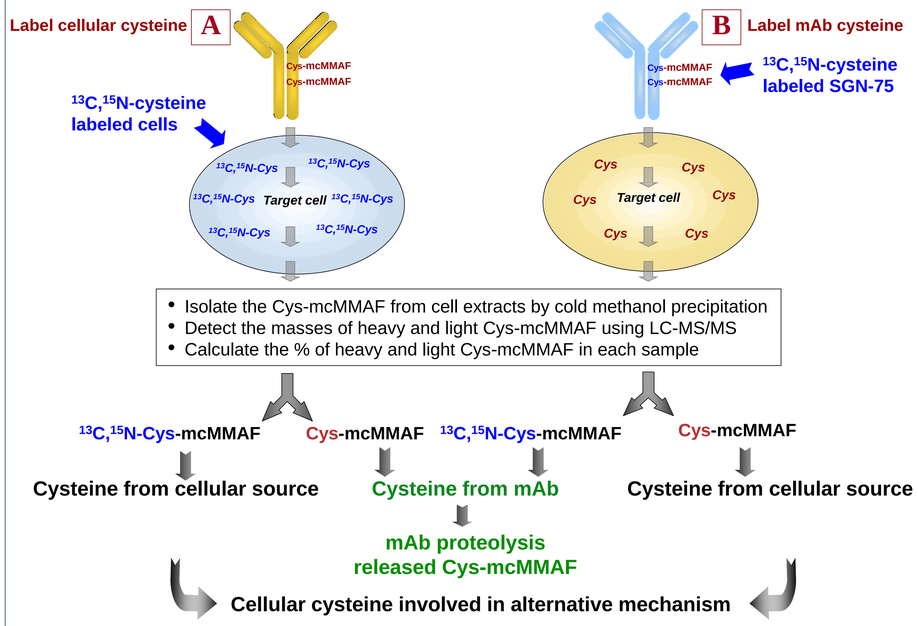
- SGN-75 is a humanized anti-CD70 monoclonal antibody (mAb) h1F6 conjugated to an average of 4 mcMMAF drug-linkers via reduced interchain disulfides
- The mcMMAF drug-linker has no built-in proteolytic cleavage mechanism for drug release
- In vitro the drug released from SGN-75 has been shown to be Cys-mcMMAF

3. Defining the Mechanism of SGN-75 Drug Release

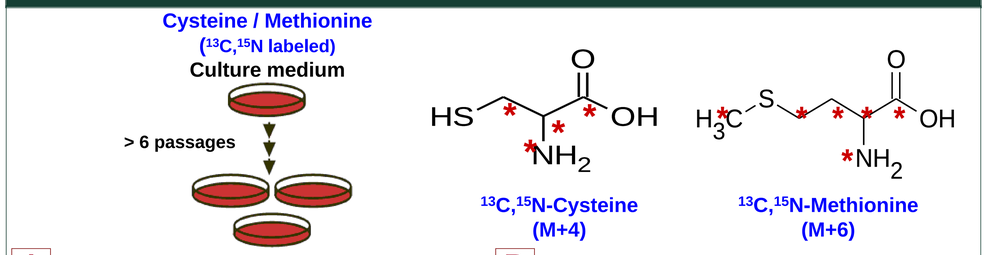


- The proposed SGN-75 drug release mechanism is lysosomal proteolysis of the mAb to release Cys-mcMMAF
- Objective: confirm the mechanism of drug release by distinguishing Cys-mcMMAF released from mAb (ADC) degradation vs. alternative mechanisms where the cysteine is from a cellular source

4. Strategies to Investigate the Mechanism of Drug Release from SGN-75



5. Labeling Cells or mAb with Heavy Isotope Cysteine



A Labeling target cell protein pool

- Labeling: grow 786-O cells (CD70+ renal cell carcinoma) in the culture medium supplemented with heavy cysteine and methionine
- Monitor: cysteine / methionine containing actin peptides isolated by gel purification and trypsinization (proxy for cellular cysteine pool)
- Incorporation efficiency: LC/MS mass shift of peptide as light Cys/Met are replaced by heavy isotope forms

B Labeling mAb

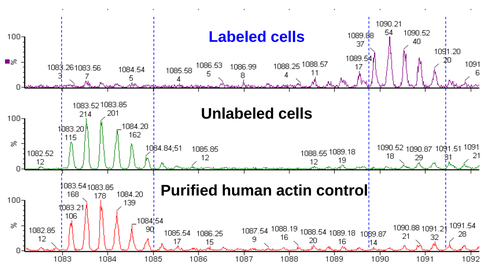
- Labeling: mAb (anti-CD70) producing CHO cells grown in culture medium supplemented with heavy isotope cysteine and methionine
- Monitor: cysteine / methionine containing tryptic mAb peptides
- Incorporation efficiency: LC/MS mass shift of peptide as light Cys/Met are replaced by heavy isotope forms

6. MS Determination of Labeling Efficiency

A Mass shift of peptide of purified actin from labeled cellular protein pool

CPEALFQPSFLGMESCGIHETTFNSIMK
(4 Da/cys, 6 Da/met)

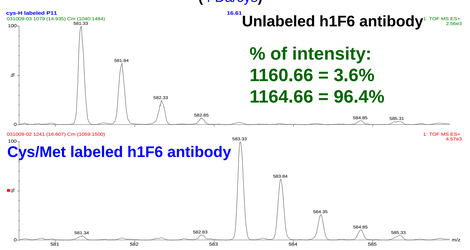
M=3246.6 (+3 ion =1083.2) M=3266.4 (+3 ion =1089.88)



- ~90% of heavy cysteines were incorporated into actin based on the signal intensity of peak shift after >10 passages

B Tryptic peptide from cysteine / methionine labeled mAb

KPGASVKVSCK (1160.66 → 1164.66)
(4 Da/cys)



- 96% of heavy cysteine was incorporated into the antibody
- Incorporation reached its maximum after 3 passages. No further incorporation was observed after 40 passages
- At least 10 Cys/Met containing peptides were analyzed and similar mass shift patterns were confirmed

7. Investigation of Released Drug in Isotope-Labeled Systems

A

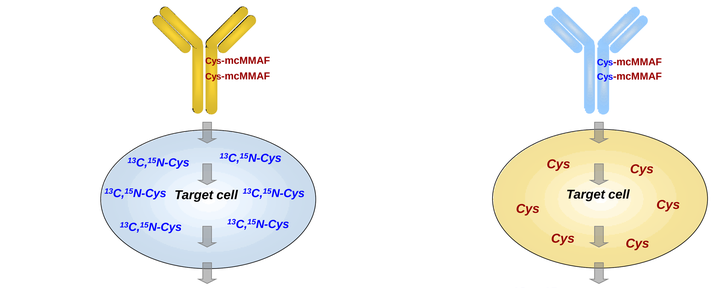
Treat 786-O cell (labeled or unlabeled) with unlabeled SGN-75 for 24 hours

Mass ID	unlabeled cells	labeled cells
Cys-mcF	100%	98%
13C,15N-cys-mcF	0%	2%

B

Treat 786-O cell with labeled or unlabeled SGN-75 for 24 hours

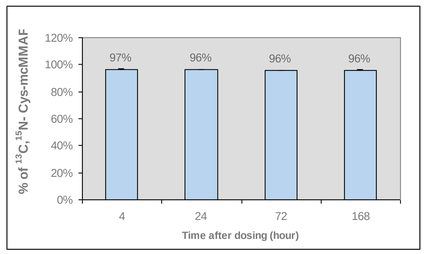
Mass ID	unlabeled SGN-75	labeled SGN-75
Cys-mcF	100%	4%
13C,15N-cys-mcF	0%	96%

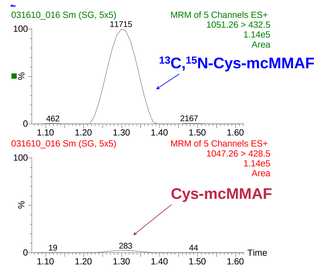


- In both systems the cysteine in the released drug matches the labeling status of the mAb
- The results suggest that cysteine in Cys-mcMMAF originates exclusively from the antibody backbone

8. Released Cys-mcMMAF from Labeled SGN-75 Treated Tumor *in Vivo*

- Heavy cysteine labeled SGN-75 was administered to mice bearing CD70 positive 786-O tumors
- Tumor samples were collected at 4 time points (n=5) after each dose
- The released drugs were extracted from methanol precipitated tumor homogenates
- The percentage of heavy and light Cys-mcMMAF was calculated by LC-MS/MS

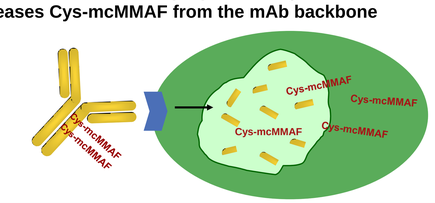




- The tumor Cys-mcMMAF was 96% in heavy isotope form
- As with the *in vitro* results, cysteine in Cys-mcMMAF originates exclusively from the antibody backbone
- The percentage of heavy Cys-mcMMAF did not change over time

9. Conclusions

- Cells were labeled with heavy isotope cysteine with >90% efficiency, and correspondingly labeled mAb was produced with 96% incorporation efficiency
- Target cells and tumor bearing mice treated with heavy cysteine labeled SGN-75 release heavy Cys-mcMMAF
- Heavy cysteine labeled CD70+ cells treated with unlabeled SGN-75 release unlabeled Cys-mcMMAF
- The results are consistent with the proposed drug release mechanism, where mAb proteolysis releases Cys-mcMMAF from the mAb backbone



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