Improving Liquid Biopsies by Boosting ctDNA Yield with Humanized Priming Agents

Julia Amaral[1]*, Fujiko Duke[1]*, Gregory Gydush[1], Ana Moscoso[1], Shervin Tabrizi[1], Viktor A. Adalsteinsson[1], J. Christopher Love[1], Eric K. Huang[1#], Carmen Martin-Alonso[1#]

1. Amplifyer Bio, Inc.

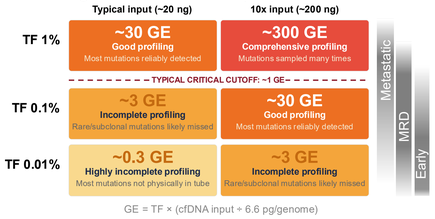
*denotes co-first #denotes co-corresponding

THE SAMPLING PROBLEM

- With typical cfDNA yields (~20 ng), the tumor genome is often stochastically sampled from a blood draw.
- This "sampling problem" – upstream of any assay - sets a ceiling on test performance and cannot be addressed with sequencing or molecular biology improvements.

➢ **Recovering more cfDNA input per blood draw would push the boundaries of what is currently attainable.**

Tumor genome equivalents (GE) per blood draw

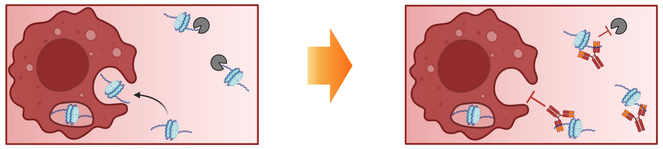


GE = TF × (cfDNA input ÷ 6.6 pg/genome)

PRIMING BOOSTS cfDNA INPUT

The biological bottleneck: cfDNA is rapidly cleared from circulation by macrophages in the liver and circulating DNases

The solution: A "**priming agent**" [1] - an engineered **DNA-binding antibody** that shields cfDNA from clearance



Here we present the **first humanized priming agents for clinical translation**, and characterize their impact on **ctDNA recovery, diagnostic performance and key cfDNA features** in murine tumor models.

CLINICAL VISION

Clinical workflow

Early detection

- ↑ **Sensitivity for early-stage cancers**

MRD

- ↑ **Sensitivity** at landmark
- Achieve Next Gen Test sensitivity with 1st Gen Test
- Allow Next Gen Tests to achieve their best LoDs
- **Shift from MRD+/- to CGP**

CGP

- ↑ **Patients** matched to **effective therapies**
- ↓ **Need for reflex** to tissue biopsy

Priming will enable liquid biopsy to be clinically actionable across cancer management

RESULTS

DESIGN AND IN VITRO CHARACTERIZATION OF HUMANIZED CANDIDATES

1. Humanization yielded numerous candidates that maintained cfDNA binding

10 parental cfDNA-binding antibodies

Parental	Species	KD MN (nM)	KD DNA (nM)	# of variants	Binder type
mAb1	Mouse	8.2	8.60	13	MN + DNA binders
mAb2	Human	4.5	5.90	3	
mAb3	Mouse	4.6	9.30	13	
mAb4	Human	4.3	0.30	3	
mAb5	Mouse	2	6.70	9	
mAb6	Mouse	1.2	19.10	6	
mAb7	Mouse	5.1	4.90	13	
mAb9	Mouse	3.1	16.50	9	
mAb8	Mouse	0.2		3	MN-only binders
mAb10	Mouse	0.5		2	

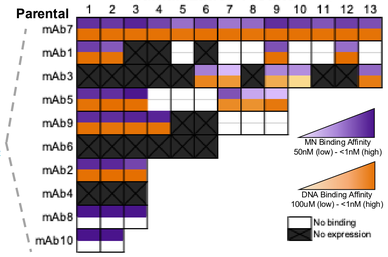
Rationally selected from the literature based on
1) Mononucleosome (MN) and DNA binding properties
2) AI-predicted developability

***In silico* humanization**

1. Germline analysis
2. Remove predicted poor expressors
3. Remove pairs with poor OAS representation.
4. Mutate liabilities

74 humanized antibodies

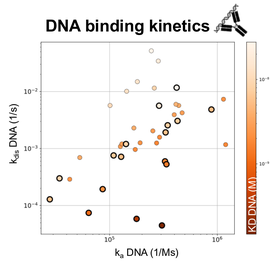
Humanization results



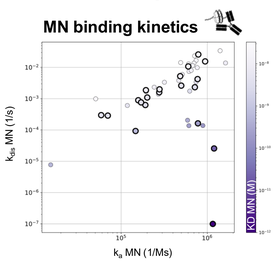
- **Expression:** 48 candidates expressed (40 in HEK293, 8 in CHO).
- **Binding:** ≥1 humanized candidates with high-affinity binding to cfDNA (<10nM) for 8/10 parentals. 39 antibodies showed binding against MN and/or DNA.

2. Many candidates demonstrate strong affinity to cfDNA

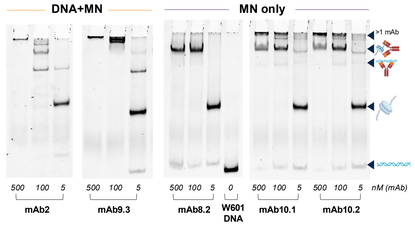
DNA binding kinetics



MN binding kinetics



Electrophoretic mobility shift assay

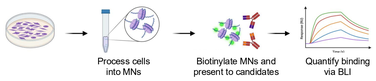


- Different binder types demonstrated different complexing ratios and shift patterns with MN.
- Some DNA+MN binders formed higher MW complexes at lower doses than others.
- One MN-only binder (mAb8) was distinct in that it only demonstrated 1:1 binding with MNs.

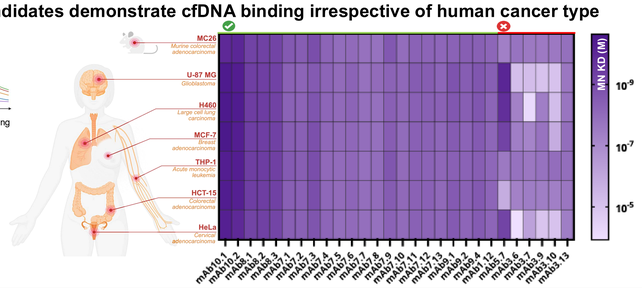
- Association (k_a) and dissociation (k_{diss}) constants for humanized candidates spanned multiple orders of magnitude across variants advanced for *in vivo* testing (bolded)

3. Most candidates demonstrate cfDNA binding irrespective of human cancer type

MN preparation and screen



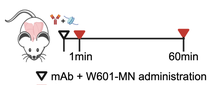
- Cell line-derived MNs were prepared using the Nucleosome Preparation Kit (Active Motif), biotinylated, and tested in Bio-Layer Interferometry (BLI) screen.
- Cell lines selected to cover most prevalent cancer types and span both low- and high-shedding tumors.



- MN-only binders demonstrate significantly higher affinity for MN than MN+DNA binders.
- Most MN+DNA binders retain single digit nM and relatively consistent affinities to MN.
- mAb5.7 showed greatest variance spanning multiple orders of magnitude.
- mAb3 variants showed more variable and lower binding affinities.

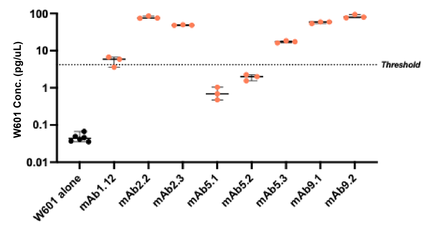
IN VIVO PRIMING PERFORMANCE

4. Most candidates improved cfDNA recovery in healthy mice

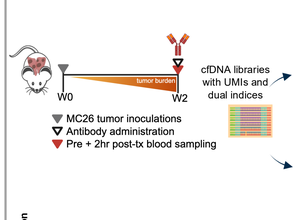


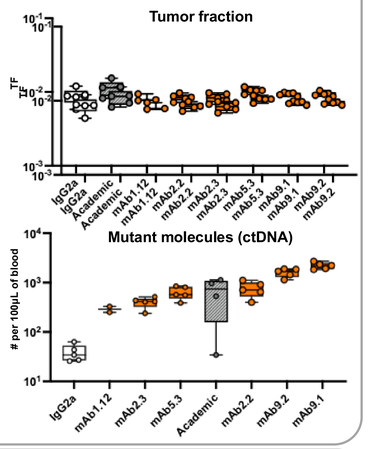
Co-injection of mAb with W601 sequence-carrying MN allows for:
- Approximation of cfDNA clearance effects, independently from shedding effects
- Quantification of cfDNA levels by qPCR

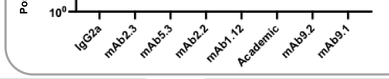
14/21 antibodies passed the threshold for testing in a murine cancer model



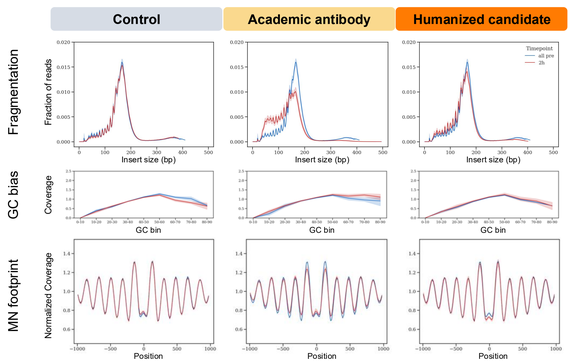
5. Most candidates improved ctDNA recovery in tumor bearing mice while preserving tumor fraction







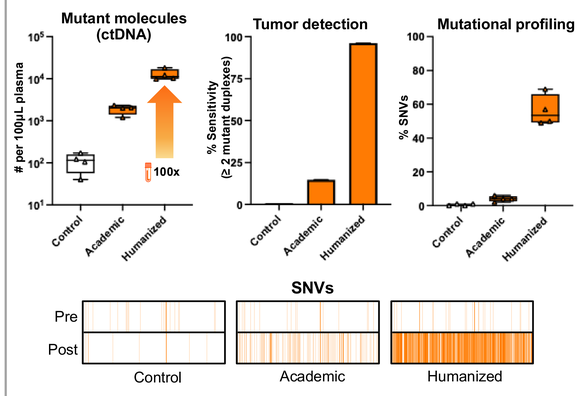
6. Some candidates better preserved key cfDNA features genome-wide



- **Coverage analysis**: *Pandepth* [2] used to calculate mean coverage as a function of GC in 1kb windows, data normalized to sample-wide mean coverage.
- **MN footprinting**: *Griffin* [3] used to calculate nucleosome patterns across CTCF (30k sites).

- Academic antibody demonstrated slight fragment shortening, but no GC bias or changes to CTCF profiles.
- Humanized candidate better preserved fragment size profiles and exhibited no GC bias or changes to CTCF profiles.

7. Some candidates achieved up to 100-fold increase in ctDNA recovery and improved diagnostic performance



- Humanized candidate achieved **up to 100x recovery** in ctDNA.
- Priming translated to significantly **better sensitivity** for cancer detection with a 16-probe panel (~96%) and **more comprehensive molecular profiling** (detecting 49-69% of SNVs post vs. 0-1% SNVs prior to priming).

CONCLUSIONS

- A diverse library of humanized DNA-binding antibodies was designed, screened, and validated in preclinical models.
- Broad binding across MNs derived from human cancer cell lines supported a generalizable mechanism of action, irrespective of tumor type.
- WGS confirmed that some candidates preserved key cfDNA fragmentomic features without introducing GC bias.
- Multiple humanized candidates outperformed the academic antibody, achieving >20× increases in ctDNA recovery two hours after administration.
  - Some candidates achieved up to a 100-fold increase in ctDNA - improving both sensitivity for disease detection and mutational profiling.
- Lead humanized candidates have been selected for potential clinical development.

References

[1] Martin-Alonso, Carmen, et al. "Priming agents transiently reduce the clearance of cell-free DNA to improve liquid biopsies." Science 383.6680 (2024): eadf2341.
[2] Yu, Huiyang, et al. "PanDepth, an ultrafast and efficient genomic tool for coverage calculation." Briefings in Bioinformatics 25.3 (2024): bbae197
[3] Doebley, Anna-Lisa, et al. "A framework for clinical cancer subtyping from nucleosome profiling of cell-free DNA." Nature communications 13.1 (2022): 7475.

Acknowledgements

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