

Optimization of Small molecules inhibitors against hnRNP A18, a regulator of protein translation and an immune checkpoint.

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ABSTRACT

We have identified the heterogenous ribonucleoprotein A18 (hnRNP A18) as a regulator of protein translation in cancer cells. hnRNP A18 recognizes a specific RNA signature motif in the 3'UTR of transcripts associated with cancer cells progression as well as CTLA-4, an immune checkpoint. Tissue micro arrays performed on human cancers indicate that hnRNP A18 is over expressed in 40 to 60% of malignant tissue as compared to normal adjacent tissue. Most importantly, down regulation of hnRNP A18 significantly reduces tumor growth in two mouse xenograft models (melanoma and breast cancer). These data indicate that hnRNP A18 is a valid target for anticancer drug development. To this Aim we first solved hnRNP A18 3D structure by NMR and crystallography and then used Computer assisted Drug design to search for potential hnRNP A18 small molecule binders targeting the RNA binding pocket. Four lead compounds that specifically target hnRNP A18 were identified. The compounds compete out hnRNP A18 RNA binding activity and specifically kill cancer cells expressing hnRNP A18 without affecting normal epithelial cells or cancer cells that do not express hnRNP A18. In vitro pharmacokinetics assay however indicate that the compounds are unstable in mouse plasma thus precluding their use into animal models. Three of the lead compounds share a common feature: they are all phenolic esters, and so it is not surprising that they have very limited half-lives in plasma. A three-pronged Medicinal chemistry strategy was then used to improve the compounds stability. First, amide congeners of the ester parent drugs were prepared, second the corresponding phenol metabolites were synthesized, and third five bulky sterically hindered esters analogues of the parent drugs were synthesized. In all, twenty derivatives were synthesized and analyzed by RNA band shift and cell viability. While most derivatives were able to compete out hnRNP A18 RNA binding activity, three analogues remain as potent or slightly better at killing cancer cells than the parent compounds. An in vitro pharmacokinetics assay was then performed on one of the ester derivatives. Our data indicate that the half-life of the ester derivative increased by ten-fold as compared to the parent compound in mouse plasma. This compound is currently being scaled up for efficacy experiments in mouse models including xenografts and syngeneic models to determine its effect on tumor immune response.

BACKGROUND

- New regulator of protein translation in cancer cells. Low or undetectable levels in normal cells. Up regulated in several human tumors
- Rapid induction in UV radiated CHO cells (1988). Human hnRNP A18 was cloned and characterized.
- hnRNP A18 protein identified in mouse following exposure to mild cold shock and is thus also known as CIRP
- Under normal physiologic conditions mostly expressed in the nucleus but translocates to the cytoplasm in response to cellular stress (UV, hypoxia:GSK3-b, CK2).

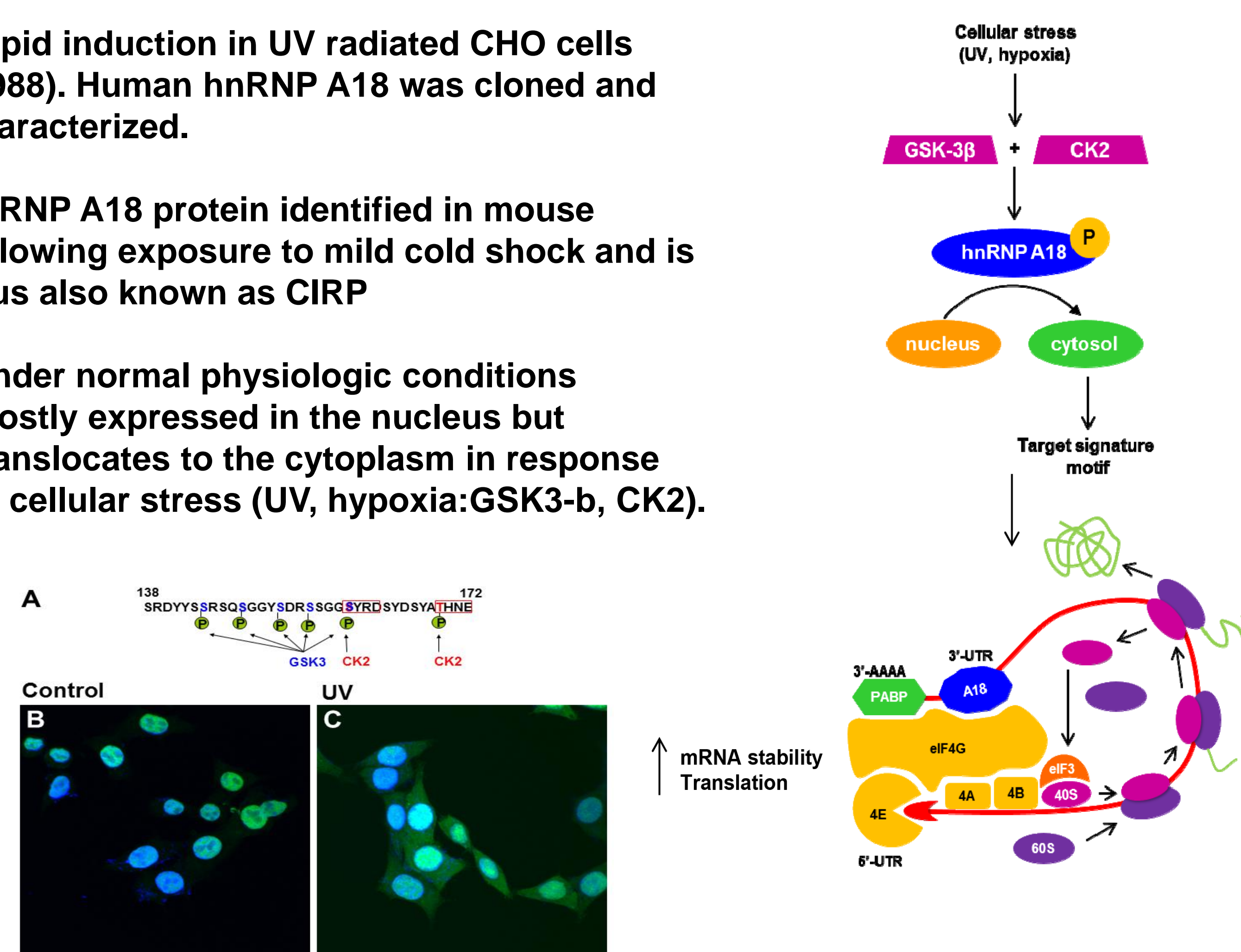


FIGURE 1: Down regulation of hnRNP A18 reduces tumor growth and metastasis

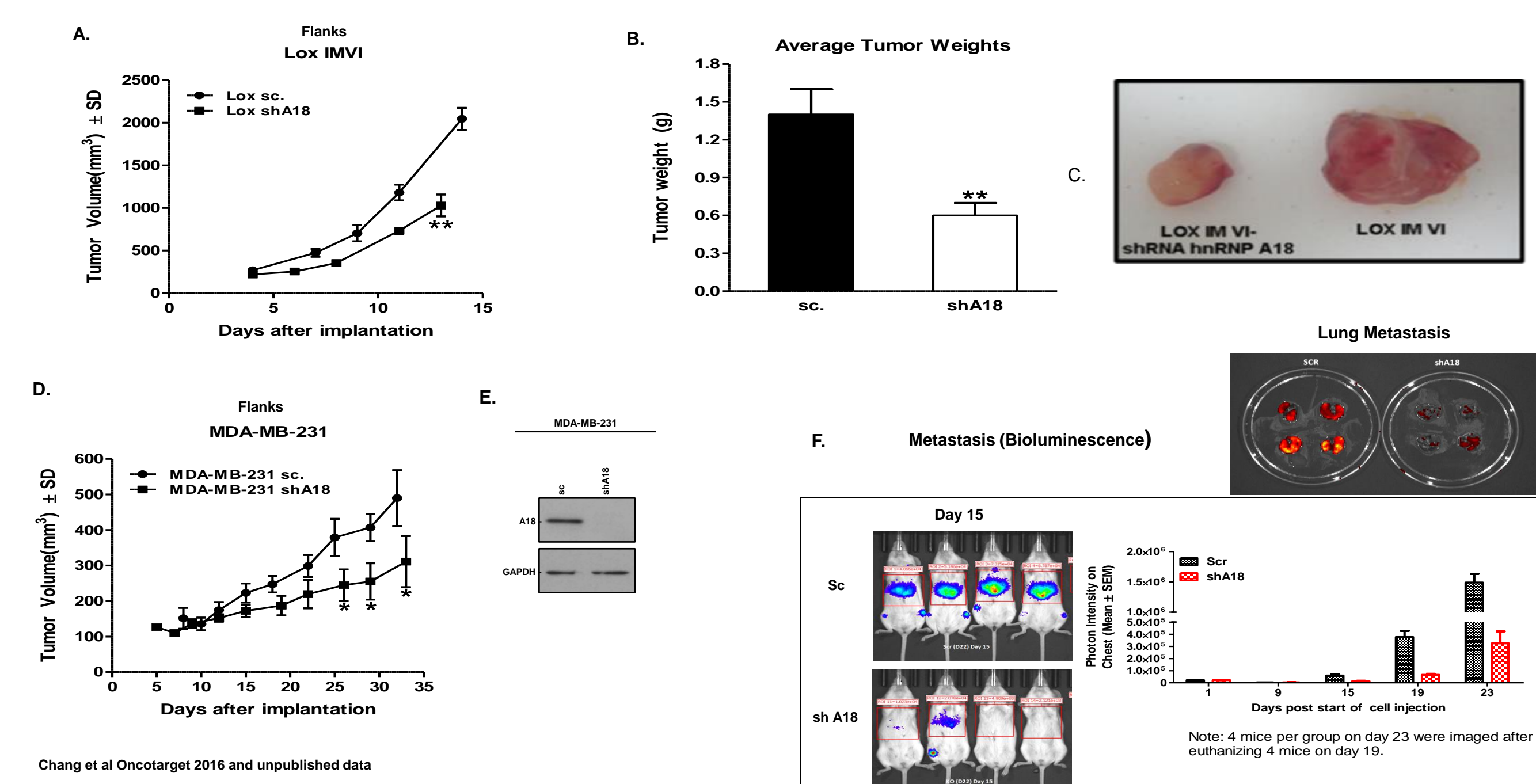


Fig.1: A-C) Downregulation of hnRNP A18 significantly reduces melanoma and breast (D) tumor growth as well as metastasis (F).

Figure 2: hnRNP A18 targets cancer progression and an immune checkpoint

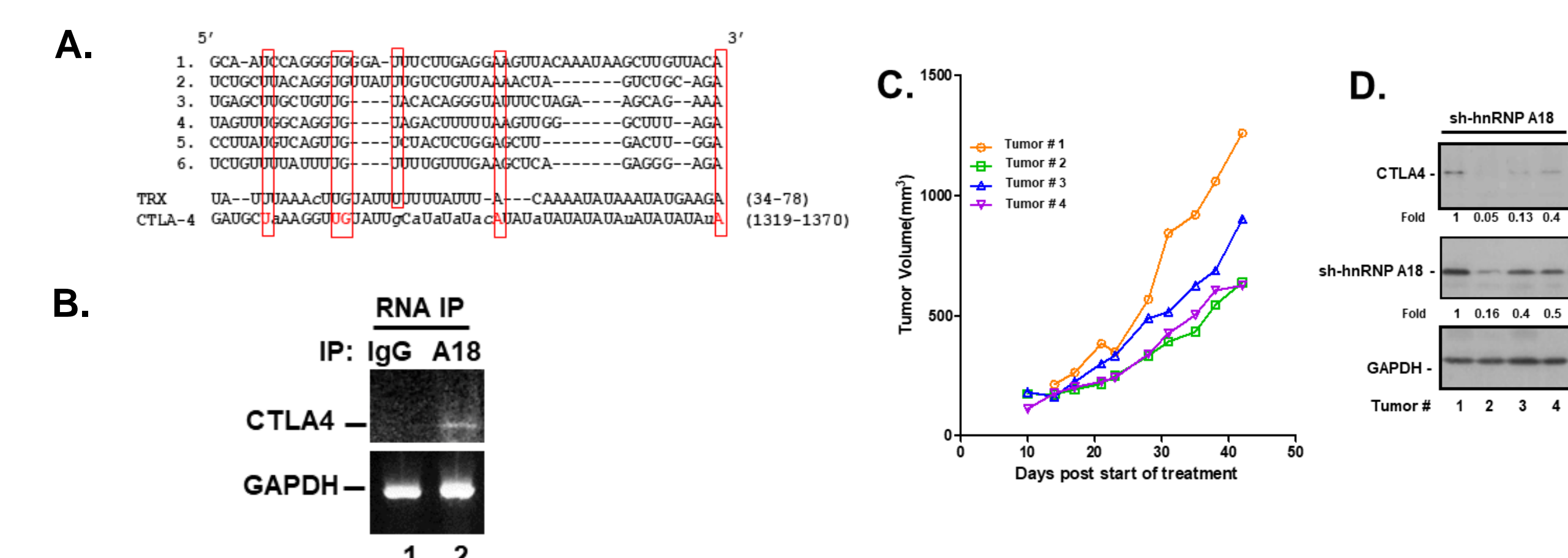


Fig.2: A) Consensus hnRNP A18 RNA binding motif and motif found in TRX and CTLA-4. B) RNA-IP Validating hnRNP A18 RNA binding motif in CTLA-4 3'UTR. C-D) Prostate tumor aggressiveness and CTLA-4 levels correspond to hnRNP A18 expression levels.

Figure 3: Site Identified Ligand Competitive Saturation

4-point pharmacophore model to perform a virtual screening (compounds >1,800,000 molecules: 154 molecules passed the initial pharmacophore filter: compounds were then ranked using the Pharmer RMSD score and Chemical Shift Perturbation (CSP) analyses (NMR))

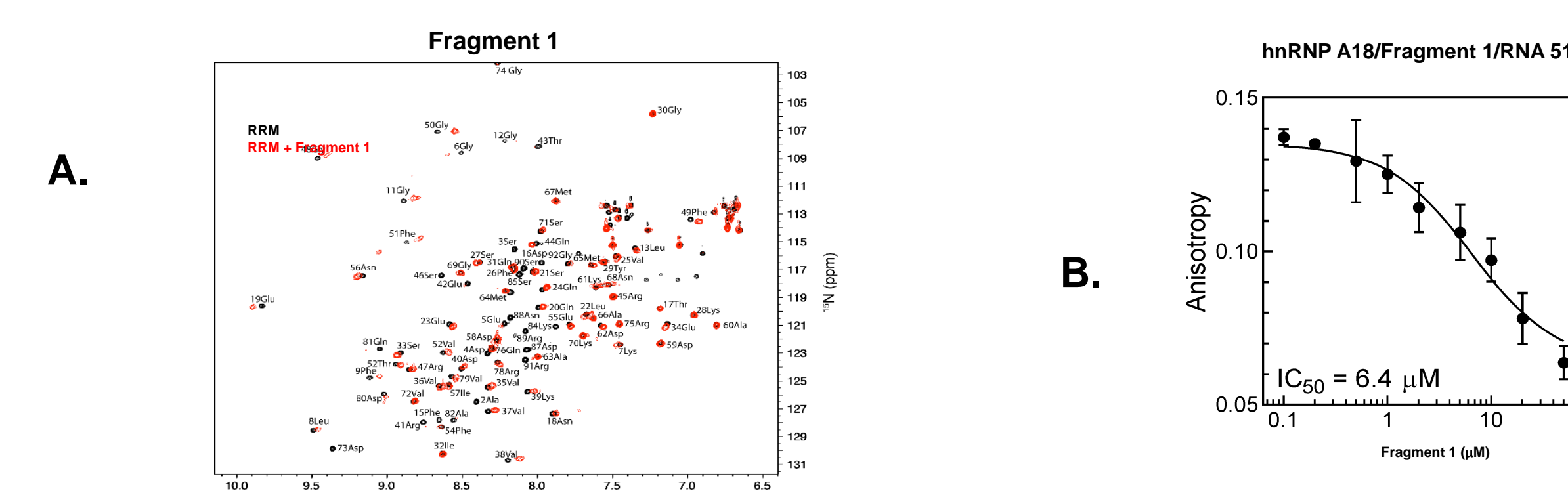


Fig.3: A) Fragment 1 directly binds to hnRNP A18 RNA binding domain as demonstrated by Chemical Shifts perturbations. [¹H-¹⁵N] TROSY-HSQC of hnRNP A18 RRM (black) overlaid with a TROSY-HSQC of hnRNP A18 with Fragment 1 (red). B) Fluorescence anisotropy obtained with hnRNP A18 full length protein and increasing amounts of Fragment 1.

Figure 4: How to target hnRNP A18 ? And drug optimization

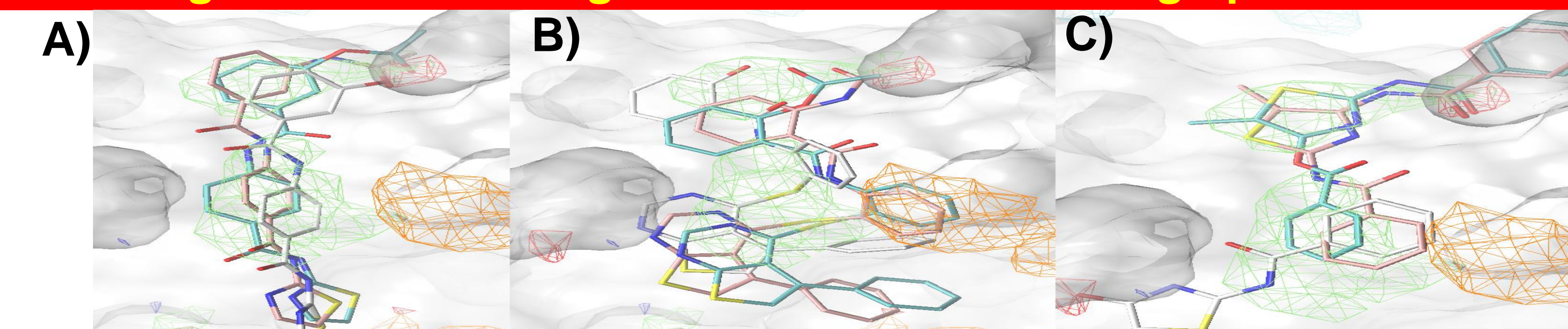


Figure 4. Binding poses from SILCS-MC "Parent Lead compounds" (A) Fragment 2, (B) Fragment 3 and (C) Fragment 4 (carbon in cyan) and their metabolites (carbon in white) and amide analogues (carbon in pink).

Table 1. LGFE for three lead compounds and their metabolites and amide analogues.

Cmpd	FRAGMENT 2	FRAGMENT 3	FRAGMENT 4
Lead	-5.12	-6.07	-5.76
Metabolite	-4.39	-6.33	-5.37

Figure 5: Parent compounds target cancer progression and CTLA-4 and derivative analogues optimization stability and half-life in mouse plasma

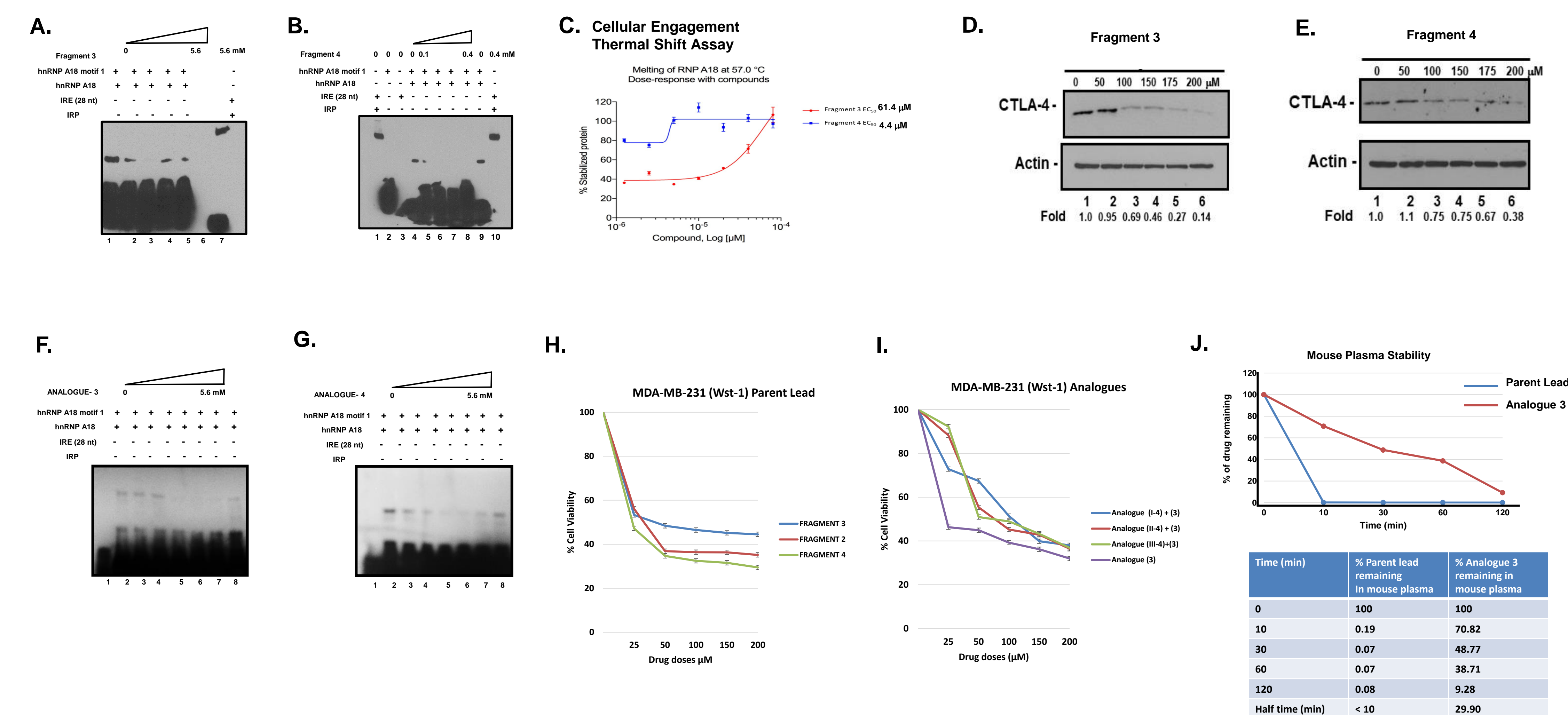


Fig.5: A-B) RNA band shifts. Fragment 3 and 4 specifically disrupt hnRNP A18 RNA binding activity. C) CETSA measuring capacity of the fragments to reach their target (hnRNP A18) in cells. D-E) Fragments 3 and 4 down regulate CTLA-4 expression. F-G) RNA band shifts. Analogue 3 and 4. H-I) Cell Viability assay (Wst-1). Parent lead and Analogues performed in MDA-MB-231 following exposure to the indicate drug, after 24 hrs. J) Mouse plasma stability comparison parent lead and their derivative analogue optimization.

CONCLUSIONS

- Identification of a new class of small molecules inhibitors (Patents pending) to specifically disrupt protein translation in cancer cells
- Fragments 3 and 4 specifically disrupt hnRNP A18 RNA binding activity *in vitro* and *in vivo*, and 4 target CTLA-4.
- We optimized the stability in plasma, from FRAGMENT 3 (2.67 min) half life to ANALOGUE 3 (29.30 min) increased 10 folds
- Medicinal chemistry, PK, PD and MTD as well as efficacy in mouse tumor xenografts are underway

ACKNOWLEDGEMENTS