#1488

Development of Anti-Nucleolin Antibodies with Broad Spectrum Anticancer Activity and Negligible Toxicity to Normal Cells

Daniel J. Fernandes[1], Laura L. Schwartz[1], Baby G. Tholanikunnel[2]

[1]CharlestonPharma, LLC, Charleston, SC

[2]Medical University of South Carolina, Charleston, SC

CharlestonPharma LLC

BACKGROUND

- We have developed a panel of fully-human monoclonal antibodies that target nucleolin.
- These HuMAbs show broad spectrum anticancer activity *in vitro*, while maintaining high antitumor selectivity *in vitro* and *in vivo*.
- The aberrant expression of nucleolin on the cell surface and in the cytoplasm of most tumor cells versus the corresponding normal tissue accounts for the broad-spectrum anticancer activity of our anti-nucleolin HuMAbs.

MECHANISM OF ACTION

Figure 1. Binding of our lead antibody, CP101.2C8, to human recombinant nucleolin. Recombinant nucleolin with an N-terminal deletion (Δ1-283) and 6x-His was purified and binding of CP101.2C8 was quantified by ELISA. The equilibrium dissociation constant was calculated to be 2.6 ± 0.7 nM S.E.M., N = 4

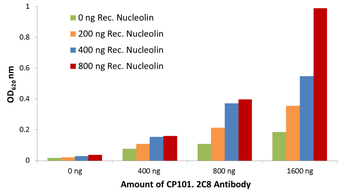
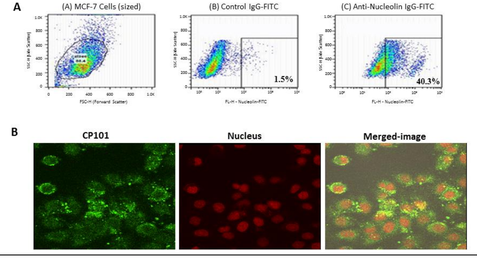
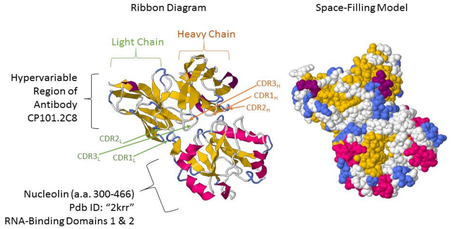


Figure 2. Cell Surface and Plasma Membrane Binding of CP101.2C8 to Cancer Cells. (A) Non-permeabilized MCF-7 cells were incubated for 1 h at room temp. with either a FITC-labeled isotype control antibody or CP101.2C8-FITC. **(B)** Non-permeabilized DU-145 prostate cancer cells were incubated for 2.5 h at 37 C. The incorporation of CP101.2C8 into the plasma membrane was determined by indirect immunofluorescence using CP101.2C8 and a FITC-conjugated secondary Ab. Nuclei were counter-stained with propidium iodide. The punctate appearance of nucleolin suggests that it was incorporated within lipid rafts in the plasma membrane.



MECHANISM OF ACTION

Figure 3. Molecular Model of the Binding of Antibody CP101.2C8R to Human Nucleolin. ClusPro 2.0 protein-protein docking software (1) was utilized in antibody mode to predict the interaction between the hypervariable region of CP101.2C8R and the structures of various nucleolin fragments in the Protein Data Bank (PDB). The most accurate model was obtained with the binding of CP101.2C8R and the RNA-binding domains 1 and 2 (RBD 1 & 2) of human nucleolin (PDB 2krr). This model is consistent with the known inhibition of nucleolin binding to the 3'UTRs of oncogenic mRNAs by anti-nucleolin antibodies (2) and the nucleolin targeting aptamer AS1411 (3).

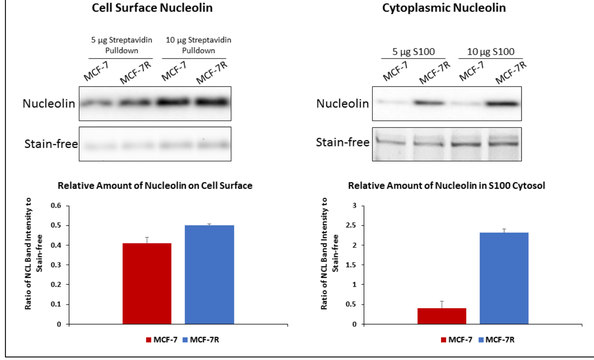


(1) Brenke et al. Application of asymmetric statistical potentials to antibody-protein docking. Bioinformatics. 2012; 28(20):2608-2614.

(2) Fogal et al. Cell surface nucleolin antagonist causes endothelial cell apoptosis and normalization of tumor vasculature. Angiogenesis. 12:91-100.

(3) Soundararajan et al. The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. Cancer Res. 2008; 68:2358-65.

Figure 4. MCF-7 Cells Resistant to CP101.2C8 Show Increased Expression of Cytoplasmic Nucleolin. MCF-7 cells were exposed to increasing concentrations of CP101.2C8 from 0.5 to 4.0 μg/ml. Western blot analysis revealed a 5.7-fold increase in S100 cytoplasmic nucleolin in MCF-7R cells compared to parental MCF-7 sensitive cells.



SELECTIVE ANTICANCER ACTIVITY

Figure 5. Effects of CP101.2C8 on the Viability of Tumor and Normal Cells. The IC50 values were determined following a 96 h exposure of the cells to antibody CP101.2C8. Cell viability was quantified using a Nexcelom Cellometer and trypan blue staining.

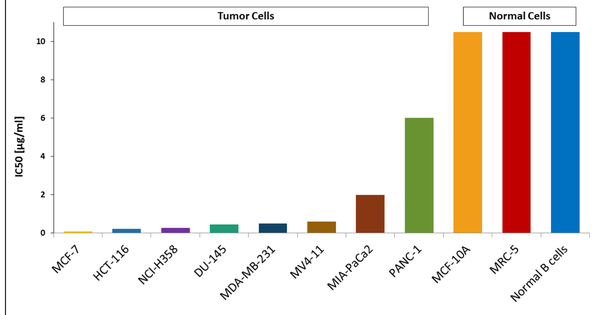
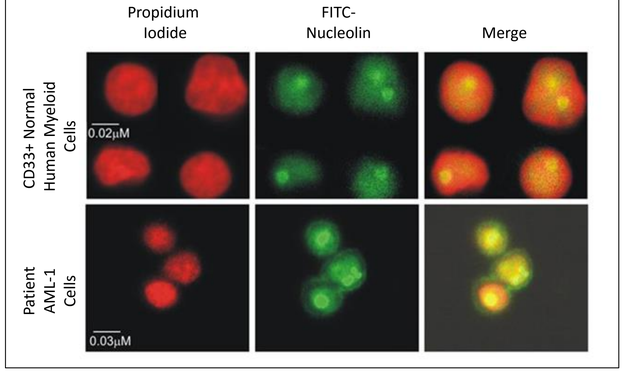


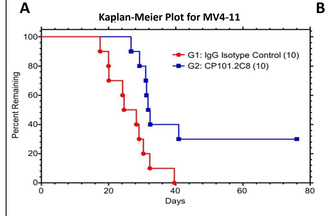
Figure 6. Aberrant Expression of Nucleolin in Patient AML Cells. In normal human myeloid cells, nucleolin staining was concentrated in nucleoli, while in patient AML-1 cells extensive nucleolin staining was observed in nuclei and in the cytoplasm/cell surface. This result along with the potent effect of CP101.2C8 in killing human MV4-11 AML cells *in vitro* (Fig. 5), provided a rationale for examining the effects of the antibody on the survival of nude mice bearing MV4-11 AML xenografts.



SELECTIVE ANTICANCER ACTIVITY

Figure 7. MV4-11 Human AML Leukemia Xenograft Model

A



B

MV4-11 Statistical Analyses	
Groups Compared	IgG Isotype Control (10) (iv) Days 1,4,7,10,13,16 vs CP101.2C8 (10) (iv) Days 1,4,7,10,13,16
Logrank (Mantel-Cox) Test	
Chi Square	7.431
df	1
P value	0.0064
P value summary	**
Are survival curves diff?	Yes
Gehan-Breslow-Wilcoxon test	
Chi Square	7.621
df	1
P Value	0.0058
P Value summary	**
Are survival curves diff?	Yes
Hazard Ratio (Mantel-Haenszel)	
Ratio	0.22
95% CI of ratio	0.08-0.61
Hazard Ratio (Logrank)	
Ratio	0.29
95% CI of ratio	0.08-0.63

ns = non-significant, $P > 0.05$
* = $P \leq 0.05$
** = $P \leq 0.01$
*** = $P \leq 0.001$

C **Safety**–CP101.2C8 was well tolerated with the only adverse event being a 16% transient loss in mean body weight. CBC was normal. Necropsy results of all major organs were normal.

CONCLUSIONS

- HuMAb CP101.2C8 and its recombinant version, CP101.2C8R, bind to cell surface nucleolin and the complex appears to utilize lipid raft mediated endocytosis for cellular entry.
- In the cytoplasm CP101.2C8 binds to RNA binding domains 1 and 2 of human nucleolin. This interferes with the stabilization of oncogene mRNAs (e.g. BCL-2, BCL-X_L) and possibly growth factor mRNAs (e.g. IL-2) by nucleolin.
- MCF-7 cells made resistant to CP101.2C8 show a 5.7-fold increase in cytoplasmic nucleolin compared to parental cells. This is consistent with increased cytoplasmic nucleolin being available for oncogene mRNA stabilization.
- CP101.2C8 has potent activity against both hematological and solid tumor cells *in vitro*, but negligible toxicity to normal cells.
- In a MV4-11 human xenograft mouse model, CP101.2C8 treatment resulted in 30% long-term survivors (Hazard ratios of 0.22-0.29) without inducing any serious toxicity to the mice.
- Nucleolin is an attractive target for anticancer drug development and CP101.2C8 is a promising therapeutic candidate. Since CP101.2C8 is internalized into tumor cells, it is being evaluated as a potential platform for ADC development.

ACKNOWLEDGEMENTS

Leukemia and Lymphoma Society (6006-06)
NCI CA109254-04S1
DoD W81XWH-12-10242
South Carolina Launch