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

Daniel J. Fernandes1, Laura L. Schwartz2, Baby G. Tholanikunnel2
1CharlestonPharma, LLC, Charleston, SC
2Medical University of South Carolina, Charleston, SC

 backgrounds:

- We have developed a panel of fully-human monoclonal antibodies that target nucleolin.
- These HuMBAs 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 HuMBAs.

MECHANISM OF ACTION

Figure 3. Molecular Model of the Binding of Antibody CP01L2C8 to Human Nucleolin. CHARMM 2 protein-protein docking software (1) was utilized in antibody mode to predict the interaction between the hypervariable region of CP01L2C8 and the structures of various nucleolin fragments in the Protein Data Bank (PDB). The most accurate model was obtained with the binding of CP01L2C8 and the RNA-binding domain 1 and 2 (RBD 1 & 2) of human nucleolin (PDB 2k9c). 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 A1411 (1). (A) Nucleolin (PDB 379U). (B) Nucleolin-RBD 1 & 2 (PDB 2k9c). (C) Anti-nucleolin aptamer A1411 (1).

Figure 4. NCI-3T3 Cells Resistant to CP01L2C8 Show Increased Expression of Cytoplasmic Nucleolin (A) NCI-3T3 cells were exposed to increasing concentrations of CP01L2C8 from 0.0 to 6.0 nM. Western blot analysis revealed a 5.7-fold increase in 120 kDa cytoplasmic nucleolin in NCI-3T3 cells compared to parental NCI-3T3 sensitive cells.

SELECTIVE ANTICANCER ACTIVITY

Figure 5. Effects of CP01L2C8 on the Viability of Tumor and Normal Cells. The %D values were determined following a 72 h exposure of the cells to antibody CP01L2C8. Cell viability was quantified using a Cleaved Caspase-3 and trypan blue staining.

Figure 6. NCI-3T3 Cells Resistant to CP01L2C8 Show Increased Expression of Cytoplasmic Nucleolin (A) NCI-3T3 cells were exposed to increasing concentrations of CP01L2C8 from 0.0 to 6.0 nM. Western blot analysis revealed a 5.7-fold increase in 120 kDa cytoplasmic nucleolin in NCI-3T3 cells compared to parental NCI-3T3 sensitive cells.

Figure 7. NCI-3T3 Human AML Leukemia Xenograft Model

CONCLUSIONS

- In the cyttoplasm CP01L2C8 binds to RNA binding domains 1 and 2 of human nucleolin. This interaction interferes with the stabilization of oncogenic mRNAs (e.g. BCL-2, BCL-1, X) and possibly growth factor mRNAs (e.g. GM-CSF, VEGF).
- MCF-7 cells made resistant to CP01L2C8 show a 5.7-fold increase in cytoplasmic nucleolin compared to parental cells. This is consistent with increased cytoplasmic nucleolin-binding available for oncoprotein stabilization.
- CP01L2C8 has potent activity against both hematological and solid tumor cells in vitro, but negligible toxicity to normal cells.
- A MV-12 human xenograft mouse model, CP01L2C8 treatment resulted in 30% long-term survivors (Hazard ratios of 0.22-0.29) without inducing any serious toxicity to the mouse.
- Nucleolin is an attractive target for anticancer drug development and CP01L2C8 is a promising therapeutic candidate. CP01L2C8 is being evaluated as a potential platform for ADC development.

ACKNOWLEDGEMENTS

Charleston Pharma Charleston, SC 29407
1150 Corporate Center Drive
Charleston, SC 29421

Nucleolin (PDB 379U). (B) Nucleolin-RBD 1 & 2 (PDB 2k9c). (C) Anti-nucleolin aptamer A1411 (1).

Figure 3. Molecular Model of the Binding of Antibody CP01L2C8 to Human Nucleolin. CHARMM 2 protein-protein docking software (1) was utilized in antibody mode to predict the interaction between the hypervariable region of CP01L2C8 and the structures of various nucleolin fragments in the Protein Data Bank (PDB). The most accurate model was obtained with the binding of CP01L2C8 and the RNA-binding domain 1 and 2 (RBD 1 & 2) of human nucleolin (PDB 2k9c). 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 A1411 (1). (A) Nucleolin (PDB 379U). (B) Nucleolin-RBD 1 & 2 (PDB 2k9c). (C) Anti-nucleolin aptamer A1411 (1).

Figure 4. NCI-3T3 Cells Resistant to CP01L2C8 Show Increased Expression of Cytoplasmic Nucleolin (A) NCI-3T3 cells were exposed to increasing concentrations of CP01L2C8 from 0.0 to 6.0 nM. Western blot analysis revealed a 5.7-fold increase in 120 kDa cytoplasmic nucleolin in NCI-3T3 cells compared to parental NCI-3T3 sensitive cells.

Figure 5. Effects of CP01L2C8 on the Viability of Tumor and Normal Cells. The %D values were determined following a 72 h exposure of the cells to antibody CP01L2C8. Cell viability was quantified using a Cleaved Caspase-3 and trypan blue staining.

Figure 6. NCI-3T3 Cells Resistant to CP01L2C8 Show Increased Expression of Cytoplasmic Nucleolin (A) NCI-3T3 cells were exposed to increasing concentrations of CP01L2C8 from 0.0 to 6.0 nM. Western blot analysis revealed a 5.7-fold increase in 120 kDa cytoplasmic nucleolin in NCI-3T3 cells compared to parental NCI-3T3 sensitive cells.

Figure 7. NCI-3T3 Human AML Leukemia Xenograft Model

CONCLUSIONS

- In the cyttoplasm CP01L2C8 binds to RNA binding domains 1 and 2 of human nucleolin. This interaction interferes with the stabilization of oncogenic mRNAs (e.g. BCL-2, BCL-1, X) and possibly growth factor mRNAs (e.g. GM-CSF, VEGF).
- MCF-7 cells made resistant to CP01L2C8 show a 5.7-fold increase in cytoplasmic nucleolin compared to parental cells. This is consistent with increased cytoplasmic nucleolin-binding available for oncoprotein stabilization.
- CP01L2C8 has potent activity against both hematological and solid tumor cells in vitro, but negligible toxicity to normal cells.
- A MV-12 human xenograft mouse model, CP01L2C8 treatment resulted in 30% long-term survivors (Hazard ratios of 0.22-0.29) without inducing any serious toxicity to the mouse.
- Nucleolin is an attractive target for anticancer drug development and CP01L2C8 is a promising therapeutic candidate. CP01L2C8 is being evaluated as a potential platform for ADC development.

ACKNOWLEDGEMENTS

Charleston Pharma Charleston, SC 29407
1150 Corporate Center Drive
Charleston, SC 29421