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Experimental and Molecular Therapeutics

## Abstract 5620: Novel phage display-derived neuroblastoma-targeting peptides potentiate the effect of drug nanocarriers in preclinical settings.

Monica Loi, Daniela Di Paolo, Marco Soster, Chiara Brignole, Alice Bartolini, Laura Emionite, Jessica Sun, Pamela Becherini, Flavio Curnis, Andrea Petretto, Monica Sani, Alessandro Gori, Claudio Gambini, Renato Longhi, Michele Cilli, Theresa M. Allen, Federico Bussolino, Wadih Arap, Renata Pasqualini, Angelo Corti, Mirco Ponzoni, Serena Marchiò, and Fabio Pastorino

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### Abstract

**Purpose:** Molecular targeting of drug delivery nanocarriers is expected to improve their therapeutic index while decreasing their toxicity. The identification of novel peptide ligands specific for cells present in high-risk neuroblastoma, a childhood tumor mostly refractory to current therapies, is needed.

**Experimental design:** We performed combined in vitro/ex-vivo phage display screenings on human neuroblastoma cell lines and on tumors derived from orthotopic mouse models of human neuroblastoma. Binding validation and homing in vivo of selected phage clones were tested by immunohistochemistry/immunofluorescent analyses. Cell association experiments in vitro with the corresponding synthetic biotin-labeled peptides were performed. In vitro cytotoxicity and in vivo tumor



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accumulation and therapeutic experiments were

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Using proper subtractive protocols, we identified phage clones specific either for the primary tumor, its metastases, or for the stromal components. Globally, we isolated 121 phage-displayed neuroblastoma-binding peptides; of these, 26 bound the primary tumor, 15 the metastatic mass, 57 and 23 their respective microenvironments. Of these, five phage clones were further validated for their specific binding ex-vivo to biopsies from stage IV neuroblastoma patients and to neuroblastoma tumors derived from mice. All five clones also targeted tumor cells and vasculature in vivo when injected into neuroblastoma-bearing mice. Coupling of the corresponding targeting peptides with doxorubicin-loaded nanocarriers led to a significant inhibition in tumor volume and enhanced survival in preclinical neuroblastoma models.

**Conclusions:** Our findings demonstrate that novel ligands of neuroblastoma-associated markers are functional in the design of nanocarriers with therapeutic efficacy paving the way to their clinical development.

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