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Characterization of a re-engineered, mesothelin-targeted *Pseudomonas* exotoxin fusion protein for lung cancer therapy

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ABSTRACT

Mesothelin overexpression in lung adenocarcinomas correlates with the presence of activating KRAS mutations and poor prognosis. Hence SS1P, a mesothelin-targeted immunotoxin, could offer valuable treatment options for these patients, but its use in solid tumor therapy is hampered by high immunogenicity and non-specific toxicity. To overcome both obstacles we developed RG7787, a de-immunized cytotoxic fusion protein comprising a humanized SS1 Fab fragment and a truncated, B-cell epitope silenced, 24 kD fragment of *Pseudomonas* exotoxin A (PE24). Reactivity of RG7787 with sera from immunotoxin-treated patients was >1000 fold reduced. *In vitro* RG7787 inhibited cell viability of lung cancer cell lines with picomolar potency. The pharmacokinetic properties of RG7787 in rodents were comparable to SS1P, yet it was tolerated up to 10 fold better without causing severe vascular leak syndrome or hepatotoxicity. A pharmacokinetic/pharmacodynamic model developed based on NCI-H596 xenograft studies showed that for RG7787 and SS1P, their *in vitro* and *in vivo* potencies closely correlate. At optimal doses of 2–3 mg/kg RG7787 is more efficacious than SS1P. Even large, well established tumors (600 mm^3) underwent remission during three treatment cycles with RG7787. Also in two

Abbreviations: ADAs, anti-drug antibodies; CI, confidence interval; Fab, antibody fragment comprising the variable and the first constant domains; Fv, antibody fragment comprising only the variable domains; h, hour(s); PDX, patient-derived xenograft; PE, *Pseudomonas* exotoxin A; RIT, recombinant immunotoxin; TGI, tumor growth inhibition; TV, tumor volume; VLS, vascular leak syndrome.

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patient-derived lung cancer xenograft models, Lu7336 and Lu7187, RG7787 showed anti-tumor efficacy. In monotherapy two treatment cycles were moderately efficacious in the Lu7336 model but showed good anti-tumor activity in the KRAS mutant Lu7187 model (26% and 80% tumor growth inhibition, respectively). Combination of RG7787 with standard chemotherapies further enhanced efficacy in both models achieving near complete eradication of Lu7187 tumors.

1. Introduction

Recombinant immunotoxins (RITs) consist of a bacterial toxin linked to an antibody fragment that is directed against a tumor-specific cell-surface antigen. While different RITs have shown promising preclinical efficacy, their clinical use has been severely hampered by immunogenicity and vascular leak syndrome (VLS) as dose-limiting toxicity (Weidle et al., 2014). The SS1P molecule developed at the National Cancer Institute (NCI) is a typical RIT consisting of a disulfide-stabilized single chain Fv fragment (dsFv) for tumor targeting, in this case derived from the SS1 mouse anti-mesothelin antibody, and a 38 kDa *Pseudomonas* Exotoxin A fragment (PE38) comprising domains II and III as the cytotoxic payload. In phase 1 trials, SS1P monotherapy given either as bolus intravenous infusion (Hassan et al., 2007) or by continuous infusion (Kreitman et al., 2009) showed only minor antitumor activity after a single treatment cycle. About 90% of treated patients developed neutralizing antibodies to SS1P after only one therapy cycle, which prohibited successful re-treatment and sustained clinical benefit (Pastan and Hassan, 2014). Hence the primary goal for generating a clinically more useful, re-engineered version of SS1P was to reduce its immunogenicity thereby allowing for multiple treatment cycles. To achieve this, the bacterial toxin portion was reduced to 24 kD consisting essentially only of domain III and the furin cleavage motif of PE. In addition seven amino acid exchanges were introduced into domain III to "silence" previously mapped B-cell epitopes (Weldon et al., 2013). The murine antibody fragment of SS1P was also humanized and in addition converted to a Fab fragment to compensate for the molecule size reduction by truncation of the toxin and prevent enhanced renal clearance. A further goal of the re-engineering effort was to reduce the adverse effect potential of the molecule, particularly with regards to causing VLS, which can result in a clinically difficult to manage combination of edema, weight gain, and hypotension (Kelly et al., 2012). An improved off-target toxicity profile should enable to clinically exploit more aggressively combination regimen with standard of care chemotherapies. We describe here the generation of RG7787, the first mesothelin-targeted RIT with substantially reduced antigenicity and improved tolerability in animals that retains cytotoxic potency and pharmacokinetic properties comparable to SS1P.

Evaluation of SS1P has primarily focused on solid tumor indications reported to show close to 100% prevalence of mesothelin positivity, like epithelial mesotheliomas (Ordonez, 2003) and pancreatic adenocarcinomas (Argani et al., 2001). Literature reports of mesothelin expression in lung adenocarcinomas suggest much lower prevalence ranging from 30% to 50%. However, since lung cancer causes globally more than 1.5 million deaths per year (Torre et al., 2015), it is an important indication for clinical development of RG7787. Moreover, for almost half of all advanced non-small cell lung cancer cases there is no targeted therapy available, particularly for the ~25% of lung adenocarcinomas, that carry a mutated KRAS allele (Kris et al., 2014). A recent immunohistochemical analysis of samples from 93 lung adenocarcinoma patients performed at the NCI found that mesothelin expression, as defined by positive staining in >25% of the tumor cells, was an independent predictor for poor survival and was strongly associated ($p < 0.0001$) with KRAS mutation status (Thomas et al., 2015). In the present study we characterized the *in vitro* and *in vivo* potency of RG7787 in lung cancer models. RG7787 showed high potency on a series of lung cancer cell lines that were preselected for mesothelin expression based on Affymetrix chip data. In efficacy studies with standard as well as patient-derived xenografts, it dose-dependently induced tumor regressions at an optimal dose of ~2.5 mg/kg. Combination with chemotherapy led to almost complete tumor eradication in a KRAS mutated lung cancer model suggesting this could be a promising therapeutic approach for a subpopulation of lung adenocarcinoma patients that currently lack targeted therapy options.

2. Material and methods

2.1. Cell lines and culture conditions

Human lung carcinoma cells NCI-H596, obtained from the NCI at the National Institute of Health (Bethesda, Maryland, USA), and AsPC-1-luc cells, a stably luciferase transfected pancreatic adenocarcinoma cell line purchased from ProQinase (Freiburg, Germany), were cultured at 37 °C with 5% CO_2 in RPMI 1640 high glucose (4.5 g/L) medium supplemented with 10% FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, and 10 mM Hepes. All other lung cancer cell lines were purchased by Horizon Discovery from commercial cell banks and cultivated exactly as described in the Cancer Cell Line Encyclopedia (CCLE) by the Broad Institute (Barretina et al., 2012). All cultured cells retained the characteristic phenotype described by the commercial providers and the CCLE. Cells were immediately expanded, multiple aliquots cryopreserved and used for no more than 20 passages after resuscitation.