Evaluation of the immunotoxin, Proxinium™ in combination with chemotherapy and radiotherapy

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ABSTRACT

Proxinium™ is a recombinant fusion protein consisting of a tumor-targeting antibody fragment fused to a truncated form of Pseudomonas exotoxin A ($ETA_{253-608}$). Proxinium™ specifically targets the epithelial cell adhesion molecule (EpCAM) that is highly expressed on a wide variety of epithelial carcinomas, including squamous cell carcinoma of the head and neck (SCCHN). Head and neck cancer accounts for approximately 5% of all newly diagnosed invasive malignancies in North America each year and is the 6th most common cancer worldwide. With a five-year survival rate less than 40%, the current prognosis for patients is poor, indicating the lack of effective treatment. Proxinium™ is currently being evaluated in a phase II/III trial as a monotherapy for SCCHN patients. To assess the potential for administration of Proxinium™ in conjunction first line standard of care therapy for SCCHN, in vitro cytotoxicity and in vivo pharmacokinetic studies were conducted to evaluate the potential antagonistic, additive, or synergistic interactions with chemo- or radiotherapy. In vitro cytotoxicity was evaluated pre-, concurrent, and post-treatment with Proxinium™ in SCCHN cell lines CAL 27 and SCC-15. Cell growth inhibition, in combination with various chemotherapeutic agents (cisplatin, carboplatin, paclitaxel, 5-fluorouracil, docetaxel, bleomycin, and methotrexate) was assessed using an MTS assay. The combination of Proxinium™ with cisplatin, carboplatin, paclitaxel, 5-fluorouracil, and docetaxel resulted in a significant additive cytotoxic effect ($p \leq 0.05$) as compared to chemotherapeutic agents administered alone. The sequence of drug administration did not influence the outcome. Growth inhibition, in combination with radiotherapy, was assessed using a clonogenic assay. The combination of Proxinium™ with radiotherapy led to a synergistic cytotoxic effect when Proxinium™ was administered after radiotherapy or additive effects when Proxinium™ was administered before or at the same time as radiotherapy. In vivo pharmacokinetic profiles generated from drug administration to Sprague-Dawley rats indicated that the pharmacokinetics of cisplatin, paclitaxel, and 5-fluorouracil was not affected when administered in combination with Proxinium™. In summary, no antagonism was observed in in vitro or in vivo studies with Proxinium™ in combination with either chemo- or radiotherapy. The additive and synergistic cytotoxic effects demonstrated in this study indicate the potential utility of Proxinium™ in conjunction with more conventional treatment modalities for patients with SCCHN.

INTRODUCTION

Proxinium™ is an anti-EpCAM scFv antibody fragment linked by a furin-cleavable peptide linker to Pseudomonas exotoxin A (ETA 252-608) that is devoid of its cell binding domain and produced as a recombinant protein in E. coli. Given the limitations of vascular leak syndrome (VLS) for ETA immunoconjugates administered IV, Viventia Biotech developed Proxinium™ for intratumoral injection for the treatment of patients with squamous cell carcinoma of the head and neck (SCCHN). Direct deposition of Proxinium™ into the tumor offers several advantages over systemically administered products. Local delivery to the tumor ensures the highest concentration of drug at the site of injection but low systemic exposure, thus minimizing the toxicity to non-target tissues. Proxinium has been shown to be a successful treatment as a single agent for advanced, recurrent SCCHN in Phase I clinical trials.

The primary treatment modalities for most patients with locoregionally advanced SCCHN are surgery where possible, and/or radiotherapy, chemo-radiotherapy or chemotherapy. Many patients experience recurrence, persistence or a second primary tumour, developing advanced disease. Locally recurrent or metastatic head and neck cancer is typically treated with systemic chemotherapy usually involving platinum-based therapy. Despite this treatment, the outcome in this patient population remains poor, with no significant improvement in survival benefit and low overall tumour response rates. Even when optimal therapy has been achieved, 30 to 50% of patients with advanced disease will develop local or regional recurrence, 20 to 30% will have metastases present and 10 to 40% will develop a second primary tumour. Poor prognosis is associated with each of these events, and a median overall survival of 4 to 6 months is the expectation.

Therefore, Proxinium™ in conjunction with first line standard of care therapy, may be of greater benefit than standard care alone and warrants investigation.

RESULTS

In vitro efficacy of Proxinium in combination with chemotherapy

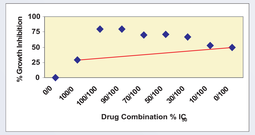
Drug-drug interactions between Proxinium and commonly used chemotherapeutics (cisplatin, carboplatin, paclitaxel, docetaxel, 5-flourouracil (5-FU), methotrexate, ifosfamide, bleomycin, cisplatin plus paclitaxel, and cisplatin plus 5-FU) were evaluated with Proxinium added pre-treatment, concurrently, and post-treatment. Potency was measured against SCCHN cell lines CAL 27 and SCC-15 using MTS assays.

Table 1. Drug combinations (% IC_{50}) used in experiments to examine *in vitro* cytotoxicity. In each experiment a fixed dose of the chemotherapeutic was used (100% of the cell line's IC_{50}) while concentrations of Proxinium varied (0 to 100% of the IC_{50} 0 to 4.0 pM).

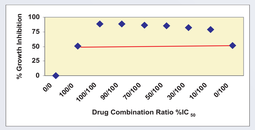
Proxinium	Chemotherapeutic
0	100
10	100
30	100
50	100
70	100
90	100
100	100
100	0

Figure 2. Treatment of Cal 27 cells with a combination of carboplatin and Proxinium resulted in significant additivity in growth inhibition. Cell viability was determined using an MTS assay. The red line indicates level of growth inhibition with single drug treatments. Values above or below the red line indicate either an additive or antagonistic effect, respectively.

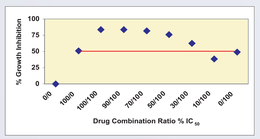
A. 24 hour treatment with carboplatin followed by a 24 hour treatment with Proxinium



B. 24 hour treatment with carboplatin together with Proxinium



C. 24 hour treatment with Proxinium followed by a 24 hour treatment with carboplatin



➢ ***Proxinium in combination with either cisplatin, carboplatin, paclitaxel, docetaxel, 5-FU, bleomycin, cisplatin & paclitaxel, or cisplatin & 5-FU showed significant additivity ($p < 0.008$)***

➢ ***Methotrexate and ifosfamide were not reactive with SCCHN cell lines CAL 27 and SCC-15***

➢ ***Drug sequence had a minimal effect, indicating that the chemotherapy agents could be administered pre-treatment, concurrently or post-treatment with Proxinium***

In vivo evaluation of combination with chemotherapy

To further examine whether Proxinium had an effect on the dynamics of chemotherapeutic drugs, an *in vivo* examination was conducted by assessing the pharmacokinetic profile in Sprague-Dawley rats dosed with both Proxinium and chemotherapeutic drugs paclitaxel, cisplatin, or 5-FU

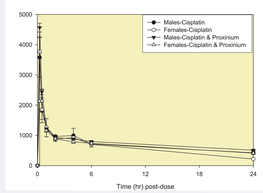
.Table 2. Examination of *in vivo* pharmacokinetic parameters indicated no difference between single therapy and combined therapy.

- Sprague-Dawley rats (4/sex/group) were administered Proxinium (77.8 µg/kg) alone or in combination with chemotherapeutic drugs paclitaxel (3 mg/kg), cisplatin (3.5 mg/kg), or 5-FU (20 mg/kg).
- Proxinium was administered via subcutaneous (SC) injection, and chemotherapeutic agents were administered via intravenous (IV) route of administration
- Blood samples were drawn over a period of 24 hours after dosing (0, 15, and 30 minutes, and 1, 2, 4, 6, and 24 hours) to evaluate drug exposure

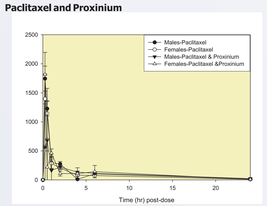
Drug	Gender	AUC_{24} ng·h/mL	Cmax ng/mL	AUC_{Inf} ng·h/mL	CL mL/kg/h	$t_{1/2}$ h	V mL/kg
Proxinium	M	491	41	543	145	4.00	6.83
	F	492	75	529	149	4.00	6.48
Cisplatin	M	16874	3578	28175	124	18.88	3174
	F	14792	3770	17922	195	9.98	2407
Cisplatin & Proxinium	M	18653	4565	37935	92	26.28	3315
	F	17092	5456	29730	118	21.38	3328
Paclitaxel	M	2916	1744	3054	982	6.59	5215
	F	2293	1396	2372	1265	6.12	5779
Paclitaxel & Proxinium	M	2449	680	2700	1111	7.73	9252
	F	2823	1818	2823	1063	4.54	5257
5-FU	M	6621	16370	6629	3017	0.20	1196
	F	5161	12486	5268	3796	0.37	1703
5-FU & Proxinium	M	6290	12478	6340	3155	0.28	1532
	F	4497	12283	4505	4439	0.22	1652

Figure 3. Comparative profiles of plasma concentration in animals administered chemotherapy (IV) alone or in combination with Proxinium (SC).

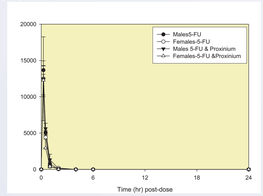
A. Cisplatin and Proxinium



B. Paclitaxel and Proxinium



C. 5-FU and Proxinium



➢ ***The combination of Proxinium with either cisplatin, paclitaxel or 5-FU did not affect the drug exposure profiles***

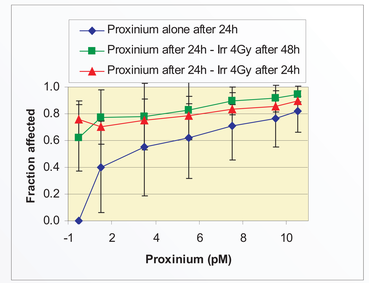
In vitro efficacy of Proxinium in combination with radiotherapy

Treatment interactions were evaluated with Proxinium administered pre-treatment, concurrently, and post-treatment with radiotherapy.

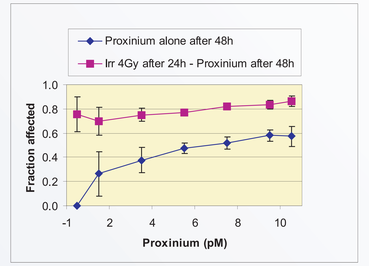
Figure 4. Treatment of SCC-15 cells with a combination of Proxinium and radiotherapy resulted in a larger proportion of cells affected by treatment.

- A single dose of radiotherapy was used, 4 Gy (100% of the cell line's IC_{50}) while concentrations of Proxinium varied (0 to 100% of the IC_{50} 0 to 4.0 pM)
- Cell lines were evaluated using clonogenic assays 7 days after treatment
- Data from three independent experiments is shown

A. 24 hours of treatment with Proxinium



B. 48 hours of treatment with Proxinium

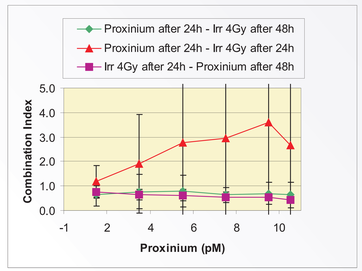


➢ ***The combination of Proxinium with radiotherapy increased the cytolytic effect against SCCHN cell lines***

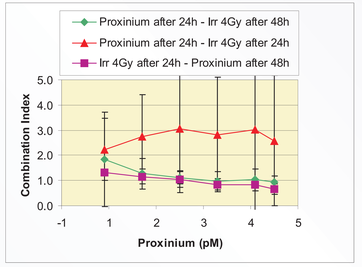
Figure 5. Treatment of SCCHN cell lines with Proxinium and radiotherapy resulted in additive or synergistic effects.

- A combination index (CI) value was used to determine whether the combination effect was additive, antagonistic, or synergistic
- CI value <1 indicates a synergistic effect
  CI value not different from 1 indicates an additive effect
  CI value >1 indicates an antagonist effect
- Data from three independent experiments is shown

A. SCC-15



B. CAL 27



Synergism was achieved:

➢ ***CAL 27: Proxinium treatment after radiotherapy with concentrations of Proximiun higher than 2 pm***

➢ ***SCC-15: Proxinium treatment before or after radiotherapy***

CONCLUSIONS

- Proxinium in combination with chemotherapy had an additive effect on overall potency against SCCHN cell lines
- Proxinium in combination with chemotherapy does not alter the in vivo pharmacokinetic profile of chemotherapeutics
- Proxinium in combination with radiotherapy had a synergistic cytolytic effect when administered before or after radiotherapy

These results clearly demonstrate the potential use of Proxinium as an adjuvant treatment in conjunction with chemotherapy or radiotherapy

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