Journal of Oncology Research
Research Article

Generic MUC1 Epitope for Targeted Alpha Therapy for Metastatic Cancer

Barry J Allen*

Faculty of Medicine, Western Sydney University, Liverpool NSW Australia.

***Corresponding Author:** Allen BJ, Faculty of Medicine, Western Sydney University, Liverpool NSW Australia. E-mail: bja1940@outlook.com

Received: 15 September, 2018**; Accepted:** 25 October, 2018**; Published:** 16 November, 2018

Abstract

The limitations of many systemic cancer therapies are that they are not potentially curable and recurrence is common. In particular, radio-immunotherapy with beta emitting radioisotopes is not curative and most vectors are cancer type specific. To overcome these limitations, new therapies are needed that are potentially curative, have minimal adverse events in humans and preferably have generic application to many cancers. These objectives could be met by targeted alpha therapy (TAT) using the C595 MAb against the cancer expression epitope (CE) of the MUC1 receptor, labeled with an alpha emitting radioisotope to form the alpha immunoconjugate (AIC).
In this paper, preclinical testing of the ^{213}Bi-C595 AIC is reviewed for prostate, ovarian and pancreatic cancers, all of which are found to express the targeted MUC1-CE epitope. We have investigated the role of this unique AIC for control of these cancers by preclinical *in vitro* and *in vivo* studies of labeling yields, stability, *in vitro* cytotoxicity, efficacy and toxicity response in preclinical TAT. Results show conclusively that normal tissues have minimal expression of the MUC1-CE epitope and that the alpha-immunoconjugate can selectively kill cancer cells *in vitro* and inhibit the development and growth of tumours *in vivo* in a dose dependent way. As such, generic targeted alpha therapy against the MUC1-CE epitope has potential for the clinical management of those cancers that express this epitope.

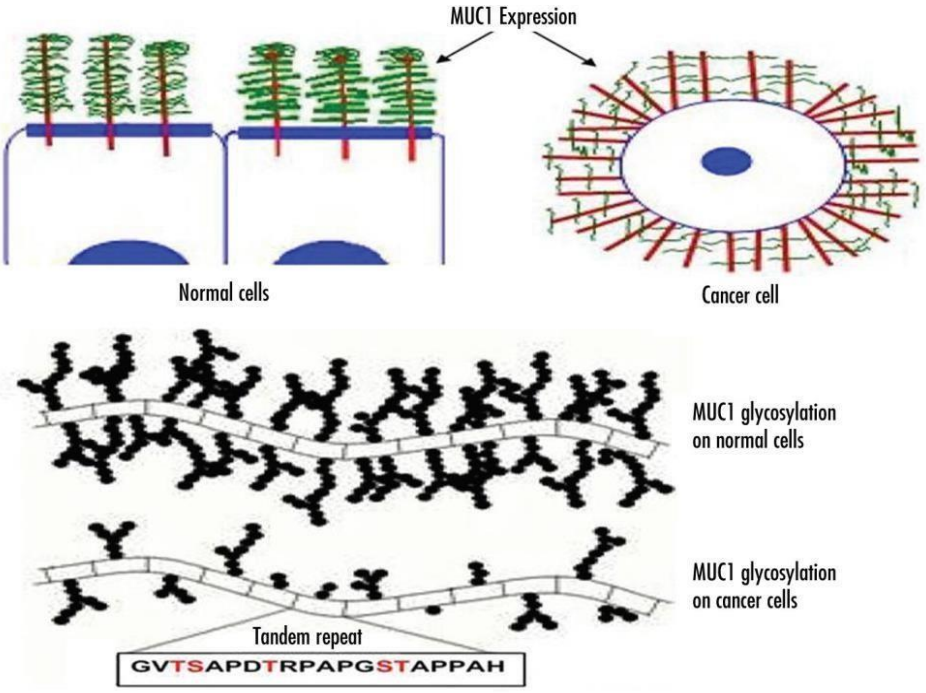
Keywords: MUC1; C595; Targeted Alpha Therapy; Prostate; Pancreatic; Ovarian Cancer

Background

Mucins line the apical surface of epithelial cells in the lungs, stomach, intestines, eyes and several other organs. They protect the body from infection by pathogen binding to oligosaccharides in the extracellular domain, preventing the pathogen from reaching the cell surface [1]. MUC1 encodes a membrane bound, glycosylated phosphoprotein. It has a core protein mass of 120-225 kDa, which increases to 250-500 kDa with glycosylation. It extends 200-500 nm beyond the surface of the cell [2]. The protein serves a protective

function by binding to pathogens and also functions in a cell signaling capacity [3]. MUC1 is overexpressed in cancers in an under-glycosylated form (Figure 1), exposing the core peptides of the extracellular domain that act as a potential target for antibody mediated therapy [3]. The highly conserved repeat of 20 amino acids varies between 20 and 125 depending on the allele. These epitopes, which comprise tandem repeats and carbohydrates present on MUC1, induce immune responses that favor targeted immunotherapy. Aberrant glycosylation also plays an important role in enhancing the internalization of MUC1 into the cytoplasm, making MUC1 a very attractive cytoplasmic delivery system for drugs and other therapeutic agents. MUC1, being present on most of the cancers of glandular epithelial origin, is a potential target for therapeutic intervention. The presence of free Bcl-2 and Bcl-xL prevents the release of cytochrome c from mitochondria, thereby preventing apoptosis.

Figure (1): MUC1 expression on normal cells and on cancerous cells [3]



The MUC1 cytoplasmic tail is shuttled to the mitochondria through interaction with hsp90. Localization of MUC1 to the mitochondria prevents the activation of apoptotic mechanisms [4] and increased expression of MUC1 in cancer increases stabilized beta-catenin. This promotes the expression of proteins that are associated with a mesenchymal phenotype, characterized by increased motility and invasiveness. In cancer cells, increased expression of MUC1 promotes cancer cell invasion through beta-catenin, resulting in the initiation of epithelial-mesenchymal transition that promotes the formation of metastases [5, 6]. As such, over-expression of MUC1 is often associated with colon, breast, ovarian, lung and pancreatic cancers [7] and blood cancers [8]. High expression of MUC1 is closely associated with cancer progression and metastasis leading to poor prognosis. MUC1 is overexpressed and under glycosylated in almost all human epithelial cells of adenocarcinoma, leading to the exposure of new peptide epitopes and oligosaccharides that serve as novel target molecules, making MUC1 an attractive and broadly applicable target molecule for cancer

therapy. The immunogenic nature of the tandem repeat domain has led to the development of a series of monoclonal antibodies that react with the epitopes in this domain

Monoclonal Antibodies (MAb)

CA 27.29 (BR 27.29) is an epitope of the antigen product of the MUC1 gene seen in breast cancer. It has enhanced sensitivity and specificity and is elevated in 30% of patients with low-stage disease and 60 to 70% of patients with advanced-stage breast cancer. However, normal tissue expression is significant [9]. The anti-MUC1 antibody C595 (NCRC48) (ab28081) [10] recognizes the tetrameric motif Arg-Pro-Ala-Pro that is repeated many times within the MUC1 protein core.

We have found that human pancreatic, ovarian and prostate cancer sections show strong staining using the C595 monoclonal antibody against this MUC1 epitope, whereas expression by normal tissues is minimal. C595 is a benign MAb. However, when chelated with the bifunctional binding molecule CHX-A" and labelled with the ^{213}Bi alpha emitter, the highly cytotoxic and targeted alpha immunoconjugate (AIC) is formed [11]. The efficacy and toxicity of this AIC is the subject of this review.

High Linear Energy Transfer (LET) radiation

The most effective radiation treatments are those that not only hit the intended target but also cause the greatest amount of lethal or non-repairable damage to DNA. LET values range from 0.3 keV/μm for betas to >100 keV/μm for alphas. Alpha particles are therefore much more effective in localized killing of targeted cells as their range is short (20-80 μm). The unique physical and radiobiological properties of alpha-particles offer the potential for more specific cancer cell killing with less damage to surrounding normal tissues. The nuclear energy deposition (specific energy) of the single alpha emitters (eg ^{213}Bi, ^{211}At) is ~1000 times that of the average beta emitter. Many *in vitro* and *in vivo* experiments and clinical trials with alpha-immunotherapy have shown marked superiority over beta-immunotherapy [12], as far fewer alpha hits of the nucleus are needed to achieve cell kill. Electron micrographs of targeted alpha treated lymphoma cells have demonstrated blebbing patterns, condensation of chromosomal material and inter-nucleosomal DNA fragmentation patterns characteristic of programmed cell death (apoptosis).

Targeted Alpha Therapy (TAT)

The important advantage of alpha radiation is the high linear energy transfer (LET) to targeted cancer cells over a short range of several cell diameters [13]. As such, alpha radiation is better suited to the treatment of microscopic or small-volume disease since their short range and high energies potentially offer more efficient and specific killing of tumour cells, while sparing distant normal cells [14]. TAT is a radio-immunotherapy technique that targets cancer cells and tumour capillary endothelial cells with an alpha emitting radioisotope. The monoclonal antibody is the targeting vector that takes the alpha emitting radioisotope to the targeted cancer cells. MAbs are raised against antigens that are over-expressed by certain cancer cells and many of these have been approved by FDA for clinical use. The cytotoxic effect of targeted alpha therapy on cancer cells has been demonstrated in both *in vitro* and *in vivo* studies and clinical trials. Monoclonal antibodies or proteins labeled with alpha emitters, such as ^{212}Bi, ^{213}Bi, ^{211}At, ^{223}Ra and ^{225}Ac, have shown remarkable effects in many *in vitro* and *in vivo* experimental models. As such, isolated cancer cells in transit and micro-metastatic or minimal residual disease can be potentially eliminated. Over the past 20 years targeted alpha therapy has progressed from *in vitro* studies through *in vivo* experiments and on to clinical trials.

The first phase I trial of a Bi^{213}-immunocojugate for acute myeloid leukemia (AML) demonstrated proof of principle for

TAT [15]. The second trial was for the intralesional injection of an AIC in 16 patients with melanoma. This trial demonstrated that skin melanomas can be regressed by a single intralesional injection without any evidence of complications [16]. Further clinical trials included a phase I/II trial for treatment of AML post-chemotherapy, a systemic trial for metastatic melanoma and glioblastoma [17-19]. Tumour regression obtained from systemic TAT in the melanoma studies was attributed to the killing of tumour capillary endothelial cells and is known as tumour anti-vascular alpha therapy (TAVAT)[20]. This is the basis for the treatment of solid tumours that over-express antigens against which specific MAbs are available.

Preclinical Studies

Prostate cancer

The distribution of MUC1 and other receptors on prostate cancer cell lines and primary and metastatic prostate cancer tissues was analyzed by immunohisto-chemistry and flow cytometer [21]. In tests of antigenic expression in three prostate cancer cell lines with different targeting vectors, C595 targeting of the CE epitope of MUC1 was found to be strongly positive in all three cell lines. Tissue microarrays were used to determine the expression of MUC1, MUC2, MUC4, MUC5AC and MUC6 in human prostate cancers and to establish changes with cancer progression [18]. Paraffin embedded resections were used from radical retro-pubic prostatectomy and transurethral resections for primary, untreated and matched lymph node metastases. Mucin MAbs were used to test for Mucin expression by histo-immunochemistry. Only MUC1-CE was overexpressed, with *58% of primary cancers and 90% of lymph node metastases but not in normal prostate or healthy tissues*. Of the primary positive tumours, 6% were Gleeson 7 or higher. MUC1-CE was found to be correlated with cancer progression, making it a preferred therapeutic target. Flow cytometry uses the light properties scattered from cells for identification or quantitative measurement of physical properties. Immunophenotyping is the analysis of heterogeneous populations of cells using labeled antibodies and other fluorophore containing reagents such as dyes and stains. Strong expression of the MUC1-CE epitope in monolayers and spheroid cultures of DU145 and LN3 prostate cancers was observed, being some 2 orders of magnitude above the background fluorescence, as shown in (Figure 2) [22]. The spheroid growth was measured for volume change and growth delay by light microscopy over a 50-day period.

Spheroids were incubated with activities of 2.2, 4.8 and 6.4 MBq/ml. Compared with control groups for 2.2 or 4.8 MBq/ml after 30 days, the volume decreased for small (80-100 μm) spheroids of DU145 by 60 and 84% and by 80 and 97% for LNCaP-LN3 cells. Cytotoxicity specific to spheroids of these prostate cancer cell lines by TAT was highly dependent on antigenic expression, concentration of radioactivity and size of spheroid. Medium size spheroids (180–200 μm) showed no change in volume for 2.2 and 4.8 MBq/ml compared with controls. The slight reduction in volume for medium size spheroids with 6.4 MBq/ml was ascribed to the short range of 80 μm, short half-life of 67 minutes and slow antibody penetration kinetics of the alpha-conjugate that reduce the efficacy of TAT with Bi^{213}. TAT was found to a potent therapeutic agent against small (80-100 μm) prostate cancer cell clusters. However, a longer lived radioisotope would be preferred for larger spheroids.

Wang [22] showed that 16 of 20 prostate cancer sections were positive for MUC1-CE, whereas there were no positive sections from 5 matched normal tissues (Table 1). These data demonstrate the excellent specificity of the C595 MAb in targeting both sections from radical retro pubic prostatectomy (RRP) and transurethral resection of the prostate (TURP). Only four of 14 RRP sections and one of six TURP sections were found to be negative. However, none of the five normal tissues were found to be positive for MUC1-CE. The specificity of C595 was found to be relatively independent of the

physiological mouse model. NOSCID mice received subcutaneous, orthotropic or intratibial injections of PC3 prostate cancer cells. Tumors were excised for immunocytochemical staining with C595. Intensity and % positive cells were similar as follows (N=5): subcutaneous model +++ 85-90%; orthotopic model ++ 65-69%; intratibial model +++ 86-92% and for cancer cell clusters ++ 76-84%. Targeting PC3 prostate cancer bone xenografts in mice, a single systemic intra-peritoneal administration of ^{213}Bi-C595 was found to retard the growth of distant bone xenografts in a concentration-dependent fashion [23]. Overall, these studies demonstrated the high expression of the MUC1-CE epitope, as detected by the C595 MAb, especially in higher grade prostate cancers.

Figure (2): Expression of MUC1-CE by C595 on prostate cancer cell lines DU 145 and LNCaP-LN3 in monolayer and spheroid cultures assessed by FACS analysis. Data are presented as blue histograms with background fluorescence (red). [22]

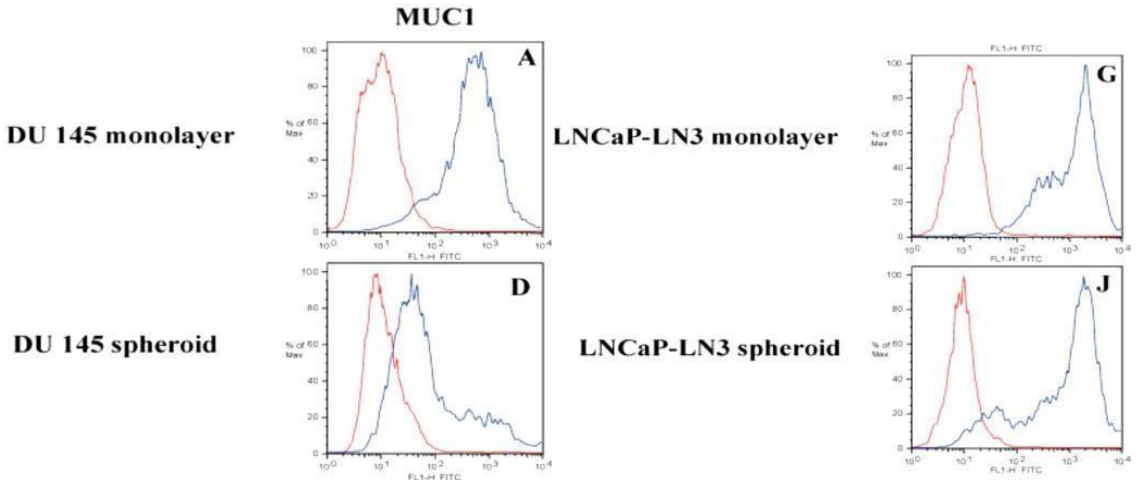


Table (1): Immunolabeling intensity of C595 MAb staining of MUC1-CE epitope [22]

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Type	RRP	RRP	RRP	RRP	RRP	RRP	RRP	RRP	RRP	RRP	RRP	RRP	RRP	RRP
Gleason	3+3	3+3	3+3	3+4	3+4	3+4	3+4	3+4	3+4	4+3	4+3	4+3	4+3	4+5
Intensity	-	++	++	+++	-	++	+++	-	++	+++	+++	+++	+	++

Sample	15	16	17	18	19	20		2	3	9	12	20
Type	TURP	TURP	TURP	TURP	RRP	RRP	matched	na	na	na	na	na
Gleason	4+5	4+5	4+5	3+4	4+3	4+4	normal	na	na	na	na	na
Intensity	++	++	++	-	+++	++		-	-	-	-	-

na: not applicable; **RRP**: radical retro pubic prostatectomy; **TURP**: transurethral resection of the prostate.
Semi-quantitative grading of immunolabeling: - (negative), + (weak), ++ (moderate), +++ (strong).

Pancreatic cancer

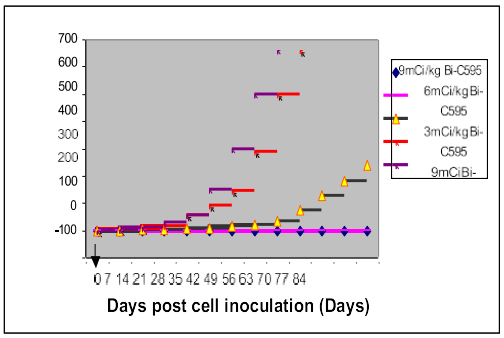
C595 was chelated with the bi-functional binding molecule CHX-A" and labelled with ^{213}Bi alpha emitter to form the AIC. The role of this AIC for the control of pancreatic cancer *in vivo* was investigated for stability, labelling yield, toxicity, cytotoxicity, response in preclinical TAT studies [11, 24]. The immunoreactivity of pancreatic cancer cell lines to C595 was determined [11]. Strong MUC1-CE expression was found for three human PC cell lines but not for the non-specific control. Epitope cell membrane expression was confirmed by confocal microscopy and by flow cytometry. These results are scored with those from flow cytometry, confocal microscopy and cell survival from incubation in the AIC. The staining intensity was scaled from positive (+) to strong positive staining (+++) and over-expression of markers is defined as being ++ or +++. *Pancreatic cancer tissues over-express (++ to +++) MUC1-CE in 81% (43/53) of patient tumors.*

In vitro incubation of pancreatic cancer cells in the AIC shows pronounced effects. The control incubations (with non-specific vector A2) gave very large D_0 values compared with those for the C595 targeting vectors; the therapeutic D_0 ratio being ~15. CFPAC-1 cultured cell clusters were incubated with the AIC, showing morphological changes, *i.e.*, clusters dissociated and cells became smaller and rounded. *Complete disaggregation was observed for ^{213}Bi-C595 at 48 hours*, whereas significant morphological changes in cell clusters were not observed for C595 alone. $^{213}BiI_5$ and the non-specific control ^{213}Bi-A2 did not exhibit apoptotic morphology. Incubation of pancreatic cancer cells in C595-cDTPA-^{213}Bi caused morphological changes of treated cancer cells and induced apoptosis. The *percentages of apoptotic cells are 77 ± 3% at 48 h after treatment* with 370 kBq/10^4 cells, compared with 12 ± 3% for the non-specific control.

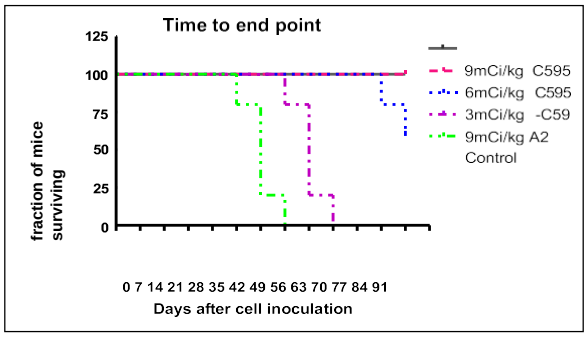
Figure (3): Systemic TAT of pancreatic cancer with C595 [24]

A: Tumour growth effect for increasing dose compared with the cold control
B: Survival time to endpoint, with complete survival for 6 mCi/kg

A



B



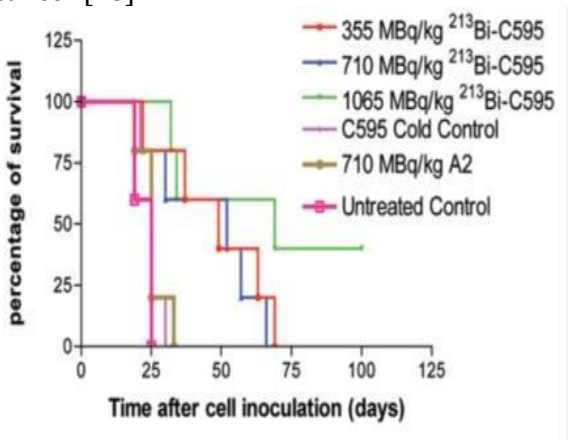
The *in vivo* regression of CFPAC-1 pancreatic cancer by local injection of the AIC [24] at the inoculation site showed complete inhibition of tumor growth for 3.7 MBq and above, while 5/5 tumors grew for both the non-specific AIC group and cold C595 control mix group after 16 weeks. Mice that received 1.85–7.4 MBq of the AIC had a median time to end-point of 112 days, which was significantly different to that for the cold C595 group (42 days) and 7.4 MBq ^{213}Bi-A2 group (74 days) ($P < 0.001$). The *In Vivo* Regression of CFPAC-1 pancreatic cancer by systemic (intraperitoneal) AIC injection of

111, 222 and 333 MBq/kg was made at two days post-inoculation of cancer cells. *Complete inhibition of tumor growth (0/5) was observed for the 222 MBq/kg and above groups, whereas 5/5 of tumors grew for non-specific AIC and cold control mix groups*. These results are shown in Figure 3A. Median post-inoculation time to the prescribed end point at 112 days for 111, 222 and 333 Bq/kg group ($P < 0.001$); P value relative to the cold C595 control mix of 42 days and non-specific AIC control of 56 days, as shown in Figure 3B. A single intraperitoneal (IP) injection of ^{213}Bi-C595 at a dose of 222 MBq/kg completely suppressed tumor growth over 16 weeks, while all control animals grew tumors. The growth inhibition of tumors and metastases was dose dependent. This means that ^{213}Bi-C595 can control tumor genesis by local or systemic TAT and can the inhibit growth of pancreatic cell clusters and pre-angiogenic lesions *in vivo.* ^{213}Bi-C595 can target and kill cancer micro-metastases, *i.e.*, cells in transit or at the pre-angiogenic stage. Therefore, multiple metastatic sites at the minimal residual disease stage should be considered the most suitable targets. The lethal pathway for the three cell lines *in vitro* after TAT was found to be predominantly by apoptosis. MUC1-CE expression in cell clusters and in a nude mouse xenograft model was found to be medium to strong tumor expression for all three cell lines, indicating that cancer cells do not lose expression for *in vitro* cell clusters and *in vivo* mouse xenografts.

Ovarian Cancer

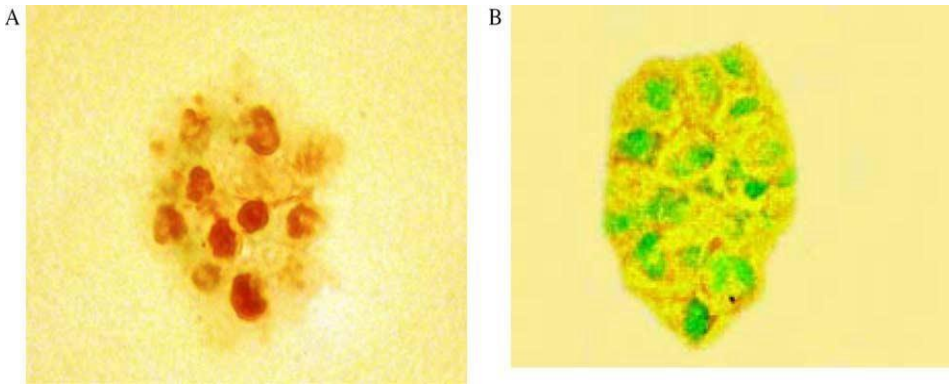
Several preclinical studies of alpha-immunoconjugates for intraperitoneal (ip) ovarian cancer with different targeting vectors and radioisotopes have been reported. The feasibility of immune-liposomal targeting of ^{225}Ac was demonstrated [25] by labeling Herceptin-immuno-liposomes (HIL) using the ionophore calcimycin. Stability and retention of the AIC was determined by incubation at 37°C in growth media. Nude mice were injected ip with SKOV3-NMP2 ovarian carcinoma cells. Ac-HIL uptake was observed primarily in the tumor and spleen, with some uptake in the peritoneal organs (kidneys, liver, stomach, and intestine). The absorbed dose and relative biological effectiveness (RBE) dose have been calculated in relevant normal tissues associated with intraperitoneal administration of ^{211}At-MX35 F(ab')2 [26]. This therapy was found to be well-tolerated for locally confined, microscopic ovarian cancer. The bladder, thyroid and kidneys received the highest estimated absorbed doses. The therapeutic effect of ^{227}Th-alpha-immunotherapy on ip growing human bioluminescent HER2 positive ovarian cancer cells has been studied [27]. The *in vitro* toxicity in bioluminescent SKOV3-luc-D3 ovarian cancer cells was assessed in a growth assay, achieving complete growth inhibition. For *in vivo* therapy, seventy female athymic nude mice received IP inoculations of cancer cells 17 days prior to injection of single ^{227}Th-trastuzumab doses of 1000 kBq/kg, 600 kBq/kg or 400 kBq/kg, or three injections with 400 kBq/kg ^{227}Th-trastuzumab separated by 4 weeks. Two control groups were given either 20 μg unlabeled trastuzumab or 0.9% NaCl. *In vivo* bioluminescence imaging was performed weekly before and after onset of therapy and tumor growth, survival and toxicity were compared.

Figure (4): Kaplan- Meier survival curves for ^{213}Bi-C595 over 100 days for ovarian cancer.[28]



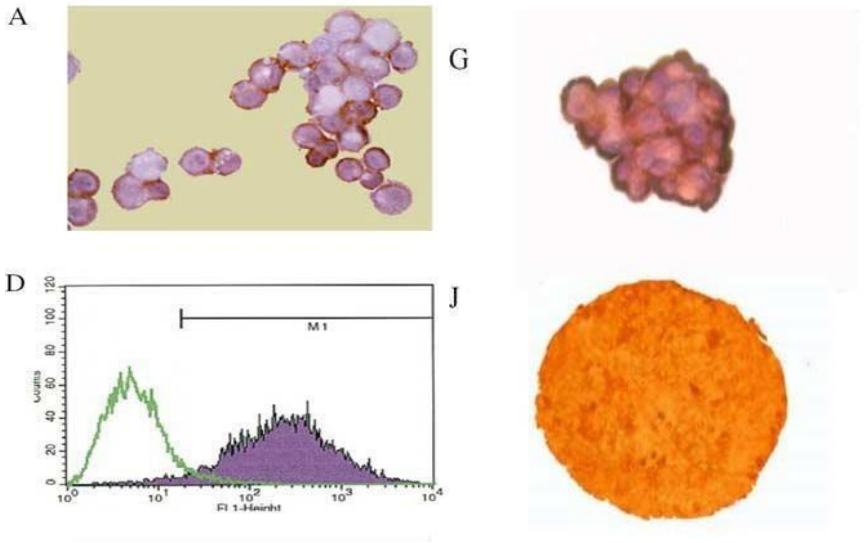
There was a statistically significant therapeutic effect of the treatment both with respect to survival and tumor growth. The maximum tolerated dosage was 600 kBq/kg, reflecting the high multiple alpha emission toxicity. IP targeted alpha therapy was clearly superior to unlabelled trastuzumab therapy. The vectors PAI2, C595 and Herceptin target the membrane-bound uPA, MUC1-CE and HER2 receptors/antigens expressed by ovarian cancer cells, respectively. The expression of these receptors in the OVCAR-3 cell line was as follows; MUC1-CE was strongly expressed, uPA moderately expressed, but HER2 was negative [29]. The alpha-emitting radionuclide Bi-213 was chelated with these targeting vectors to form alpha conjugates (AICs), the cytotoxicity of which were tested with OVCAR-3 cells. The PAI2 and C595 ACs were highly cytotoxic to the ovarian monolayer cancer cells and cell clusters in a concentration dependent fashion and cause morphological changes of treated cancer cells, inducing apoptosis.

Figure (5): TUNEL assay for OVCAR-3 spheroids; A treatment and B no treatment [29]



The efficacy, bio distribution and long-term toxicity of targeted alpha therapy were investigated for an ovarian ascites mouse model [28]. ^{213}Bi was selected for this study because of its ready availability from the Ac:Bi generator, its short half- life of 46 minutes and high linear transfer energy alpha emission makes it a suitable option for the treatment of ovarian ascites. The MUC1-CE epitope was over-expressed *in OVCAR- 3 mice ascites cells, in 73% (19/26, n = 26) of ovarian tumour sections and 5/11 matched omentum metastases, while no immunoreactivity was found with isotype control and in normal ovarian tissues.* At 9 days post-cancer cell inoculation in mice, a single ip injection of 355 MBq/kg of ^{213}Bi-C595 prolonged survival by 45 days compared with the controls (Figure 4). The highest dose administered was 1065 MBq/kg, "giving 50% " survival at 70 days. A high tumor: blood ratio (5.8) was found in the bio distribution study. Apoptotic cells were found in spheroids (Figure 5) after treatment (A) while no apoptotic cells were found in control (B). Typical apoptotic cells with condensed or fragmented nuclei are observed in treated cells, while cells without treatment show normal shape. Strong expression of MUC1-CE in OVCAR-3 monolayer cultured cells was confirmed by immunohistochemistry. Flow cytometry confirmed viable OVCAR-3 cells with 98% expression of MUC1. There was strong expression of the epitope in OVCAR-3 cell clusters (40 mm diameter) and in cultured spheroids (150 mm) [29]. Ovarian cancer cells express the targeted epitope MUC1-CE and *in vivo* systemic TAT is effective in inhibiting the development of ovarian cancer in an ascites mouse model.

Figure (6): Expression of MUC1-CE in OVCAR-3 monolayer cultured cells: (A) confirmed by immunohistochemistry and (D) Flow cytometry; (G) Expression in OVCAR-3 cell clusters (40 mm diameter) and (J) cultured spheroids (150 mm) [29].



Maximum Tolerance Doses (MTD)

Animal models give some guidance as to the toleration doses for humans but cannot replace the phase I trial. Toxicity may depend on the targeting vector and its character (murine or humanized). MTDs are given in terms of body weight, assumed to be 70 kg in humans, when not stated. The MTD value for the AIC was measured in mice [24]. Changes in mouse bodyweight with time were observed for intraperitoneal injections of 222, 296 and 370 MBq/kg of AIC, using the CHX-A" chelator. Clearance of activity was by renal filtration. Test mice suffered a small, short-term weight loss before embarking on a growth rate similar to that for the controls for over ten weeks. The maximum tolerance dose for ^{213}Bi-C595 for weight loss was found to exceed 370 MBq/kg. The maximum short-term body weight loss was 8% at ~ one week in the high dose group (370 MBq/kg). Growth rates for treated mice were independent of injected activity and were similar to controls from 2 to 7 weeks, after which the treated mice showed reduced growth rates. The maximum tolerance dose was found to exceed 1180 MBq/kg up to 21 weeks, being comparable to the efficacious dose [28].

However, the long term, delayed radiation damage to the kidneys was not investigated.

Conflict of interest

The author has a vested interest in the C595 monoclonal antibody for cancer imaging and therapy from Cancer Research UK and in a patent for targeted alpha therapy against the MUC1 epitope.

Translational Relevance

This paper reviews the evidence for targeted cancer therapy against a unique cancer expression (CE) epitope expressed on the MUC1 receptor. MUC1 is found on many epithelial tissues and is also associated with cancer progression. There is strong expression of the MUC1-CE epitope in prostate, pancreatic and ovarian cancers but not in normal tissues. As such, targeted alpha therapy against this epitope, using the C595 MAb labeled with the alpha emitting radioisotope Bi^{213}, demonstrates high cytotoxicity *in vitro* and tumour growth inhibition and regression *in vivo* within the murine tolerance dose. These results suggest that clinical therapy should be both safe and efficacious. These data provide the rationale

for phase 1 and 2 clinical trials to determine the maximum tolerance dose in humans and the therapeutic efficacy at that dose.

Conclusion

The collective studies reviewed in this paper show that the MUC1-CE epitope is strongly expressed in prostate, pancreatic and ovarian cancers. Further, ^{213}Bi labeled C595 can target the MUC1-CE epitope and inhibit prostate, pancreatic and ovarian cancer cell proliferation both *in vitro* and *in vivo*, the latter effect being within the murine tolerance dose. These results provide the basis for a new, generic, targeted therapy for the management of patients with epithelial cancers that over-express this generic epitope.

Acknowledgements

I am grateful to Syed Rizvi, Emma Song, Chang Fa Qu and Yong Li for their essential contributions to this study. Their work was supported in part by grants from the USDOD. The Institute for Transuranium Elements (ITU) provided of the Ac: Bi generators and Cancer Research UK the license for the C595 monoclonal antibody, developed by the University of Nottingham.

References

1. Moncada DM, Kammanadiminiti SJ, Chadee K (2003) Mucin and Toll-like receptors in host defense against intestinal parasites. Trends Parasitol; 19 (7): 305-311.
2. Brayman M, Thathiah A, Carson DD (2004) MUC1: a multifunctional cell surface component of reproductive tissue epithelia. Reprod Biol Endocrinol; 2(4).
3. Singh R, Bandyopadhyay D (2007) MUC1: A target molecule for cancer therapy, Cancer Biol Ther; 6(4): 481-486.
4. Yamamoto M, Bharti A, Li Y, et al. (1997) Interaction of the DF3/MUC1 breast carcinoma-associated antigen and beta-catenin in cell adhesion. J Biol Chem; 272 (19): 12492-12494.
5. Schroeder JA, Adriance MC, Thompson MC, et al. (2003) MUC1 alters beta-catenin-dependent tumor formation and promotes cellular invasion. Oncogene; 22(9): 1324-1332.
6. Roy LD, Sahraei M, Subramani DB, et al. (2011) MUC1 enhances invasiveness of pancreatic cancer cells by inducing epithelial to mesenchymal transition. Oncogene; 30(12): 1449-1459.
7. Gendler SJ (2001) MUC1, the renaissance molecule. J Mammary Gland Biol Neoplasia; 6(3): 339-353.
8. Brossart P, Schneider A, Dill PE et al. (2001) The epithelial tumour antigen MUC1 is expressed in hematological malignancy and is recognized by MUC1-specific cytotoxic T-lymphocytes. Cancer Res; 61(18): 6846-6850.
9. Engelstaedter V, Heublein S, Schumache AL et al. (2012) Mucin-1 and its relation to grade, stage and survival in ovarian carcinoma patients. BMC Cancer; 12: 600.
10. Price MR, Rye PD, Petrakou E, et al. (1998) Summary report on the ISOBM TD-4 Workshop: analysis of 56 monoclonal antibodies against the MUC1 mucin. Tumour Biol; 19(Suppl 1): 1-20.
11. Qu CF, Li Y, Song YJ, et al. (2004) MUC1 expression in primary and metastatic pancreatic cancer cells for *in vitro* treatment by (213)Bi-C595 radioimmunoconjugate. Br J Cancer; 91(12): 2086-2093.
12. Marcu LG, Bezak E, Allen BJ (2018) Global comparison of targeted alpha and targeted beta therapy for cancer: in vitro, in vivo and clinical trials. Crit Rev Oncol Haematol; 123: 7-20
13. Mulford DA, Scheinberg DA, Jurcic JG (2005) The promise of targeted alpha-particle therapy. J Nucl. Med; 46(Suppl 1): 199S-204S.
14. Allen BJ (2011) Future prospects for targeted alpha therapy. Curr Radiopharm; 4(4): 336-342.
15. Jurcic JG, Larson SM, Sgouros G, et al. (2002) Targeted alpha particle immunotherapy for myeloid leukemia. Blood; 100(4): 1233-1239.

16. Allen BJ, Raja C, Rizvi SMA, et al. (2005) Intraregional targeted alpha therapy for metastatic melanoma. Cancer Bio Ther; 4 (12): 1318-1324.
17. Raja C, Graham P, Rizvi SMA, et al. (2007) Interim analysis of toxicity and response in Phase 1 trial of systemic targeted alpha therapy for metastatic melanoma. Cancer Biol Ther; 6(6): 846-852.
18. Allen BJ, Singla AA, Abbas Rizvi SM, et al. (2011) Analysis of patient survival in a Phase I trial of systemic targeted alpha-therapy for metastatic melanoma. Immunotherapy; 3(9): 1041-1050.
19. Cozzi PJ, Wang J, Delprado W, et al. (2005) MUC1, MUC2, MUC4, MUC5AC and MUC6 expression in the progression of prostate cancer. Clin Exp metastases; 22(7): 565-573.
20. Allen BJ, Raja C, Rizvi SMA, et al. (2007) Tumour anti-vascular alpha therapy: a mechanism for the regression of solid tumours in metastatic cancer. Phys Med Biol; 52(13): L15-9.
21. Li Y, Rizvi SMA, Brown J, et al. (2004) Antigenic expression of human prostate cancer cell lines and primary prostate cancer sections for in vitro multiple targeted alpha therapy with Bi-213 conjugates. Int J Radiat Oncol Biol Phys; 60(3): 896-908.
22. Wang J, Rizvi SMA, Madigan MC, et al. (2006) Control of prostate cancer spheroid growth using 213Bi-labeled multiple targeted alpha immuno-conjugates. Prostate; 66(16): 1753-1767.
23. Li Y, Song E, Rizvi SMA, et al. (2009) Inhibition of micro metastatic prostate cancer cell spread in animal models by ^{213}Bi-labelled multiple targeted α radioimmunoconjugates. Clin Cancer Res; 15(3): 865-875.
24. Qu CF, Song YJ, Li Y, et al. (2005) In vivo and *In vitro* Inhibition of Pancreatic Cancer Growth by Targeted Alpha Therapy using ^{213}Bi-CHX.A"-C595. Cancer Biol Ther; 4(8): 848-853.
25. Har-el Y, Prideaux A, Song H, et al. (2008) Actinium-225 treatment of ovarian cancer metastases. J Nucl Med; 49(1): 140.
26. Cederkrantz E, Andersson H, Bernhardt P, et al. (2015) Absorbed Doses and Risk Estimates of At-MX35 F(ab') in Intraperitoneal Therapy of Ovarian Cancer. Int J Radiat Oncol Biol Phys; 93 (3): 569-576.
27. Heyerdahl H, Abbas N, Sponheim K, et al. (2013) Targeted alpha therapy with ^{227}Th-trastuzumab of intraperitoneal ovarian cancer in nude mice. Curr radiopharm; 6(2): 106-116.
28. Song EY, Qu CF, Rizvi SMA, et al. (2008) Bismuth-213 radioimmunotherapy with C595 anti–MUC1 monoclonal antibody in an ovarian cancer ascites model. Cancer Biol Ther; 7(1): 76-80.
29. Song YJ, Qu CF, Rizvi SMA, et al. (2006) Cytotoxicity of PAI2, C595 and Herceptin vectors labeled with the alpha emitting radioisotope Bismuth 213 for ovarian cancer cell monolayers and clusters. Cancer Letters; 234(2): 176-183.
30. Zalutsky MR (2005) Current status of therapy of solid tumours: brain tumour therapy. J Nucl. Med; 46(Suppl 1): 1515-1565.