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 over expressed 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. 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 over expressed 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 are minimal. C595 is a benign MAb. However, when chelated with the bifunctional binding molecule CHX-A and labelled with the 213Bi alpha emitter, the highly cytotoxic and targeted Alpha Immune Conjugate (AIC) is formed [11]. The efficacy and toxicity of this AIC is the subject of this review. (Figure 1) [3].

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 emitter (eg 213Bi, 211At) is ~1000 times that of the average beta emitter. Many in vitro and in vivo experiments and clinical trials with alpha-emitters have shown marked superiority over beta-emitter. Many in vitro and in vivo experiments and clinical trials with alpha-emitters 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 labelled with alpha emitters, such as 213Bi, 211Bi, 211At, 225Ra and 225Ac, 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 to clinical trials.

The first phase I trial of TAT 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 immune histochemistry and flow cytometry [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 [22]. 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 over expressed, 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.

Expression of the MUC1-CE epitope in monolayer's and spheroid cultures of DU145 and LN3 prostate cancers was some 2 orders of magnitude above the background fluorescence in Figure 2 [23]. Spheroids were incubated with different activities and spheroid growth was measured for volume change and growth delay by light microscopy over a 50-day period. 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.

TAT is a potent therapeutic agent against prostate cancer cell clusters. Targeting PC3 prostate cancer bone xenografts in mice, TAT showed that a single systemic intra-peritoneal administration of $^{213}$Bi-C595 retarded the growth of distant bone xenografts in a concentration-dependent fashion [24].

(Figure 2) Expression of MUC1 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) [23].

[23] Showed that 16 of 20 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 and transurethral resection of the prostate. (Table 1) [23].

Table 1: Immunolabeling intensity of C595 MAb staining of MUC1-CE epitope.

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NA: Not Applicable; RRP: Radical Retropubic Prostatectomy; TURP: Transurethral Resection of the Prostate.

The specificity of C595 was found to be relatively independent of the physiological mouse model [24]. NOSCID mice received subcutaneous, orthotopic or intratibial injections of PC3 prostate cancer cells. Tumours 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%.

Overall, these studies demonstrated the high expression of the MUC1-CE epitope, as detected by the C595 MAb, especially in higher grade prostate cancers. In vivo studies of TAT showed efficacy within the tolerance dose for mice.

Pancreatic cancer

C595 was chelated with the bifunctional 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 [11,25]. The immune reactivity 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 tumours.
In vitro incubation of pancreatic cancer cells in the AIC shows pronounced effects. The control incubations (with non-specific vector A2) gave very large D0 values compared with those for the C595 targeting vectors; the therapeutic D0 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^Bi-I5 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/104 cells, compared with 12 ± 3% for the non-specific control.

The in vivo regression of CFPAC-1 pancreatic cancer by local injection of the AIC [25] at the inoculation site showed complete inhibition of tumour growth for 3.7 MBq and above, while 5/5 tumours 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 tumour growth (0/5) was observed for the 222 MBq/kg and above groups, whereas 5/5 of tumours 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 ^211^Bi-C595 at a dose of 222 MBq/kg completely suppressed tumour growth over 16 weeks, while all control animals grew tumours. The growth inhibition of tumours and metastases was dose dependent. This means that ^211^Bi-C595 can control tumourigenesis by local or systemic TAT and can inhibit growth of pancreatic cell clusters and pre-angiogenic lesions in vivo. ^211^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 tumour expression for all three cell lines, indicating that cancer cells do not lose expression for in vitro cell clusters and in vivo mouse xenographs (Figure 3) [25].

**Ovarian Cancer**

Several preclinical studies of alpha-immunoconjugates for Intrapertitoneal (ip) ovarian cancer with different targeting vectors and radioisotopes have been reported. The feasibility of immuno-liposomal targeting of ^225^Ac was demonstrated [26] by labelling Herceptin-Immunon-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 tumour 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 [27]. 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 [28]. 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 unlabelled trastuzumab or 0.9% NaCl. In vivo bioluminescence imaging was performed weekly before and after onset of therapy and tumour growth, survival and toxicity were
compared. There was a statistically significant therapeutic effect of the treatment both with respect to survival and tumour 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 and HER2 receptors/antigens expressed by ovarian cancer cells, respectively. The expression of these receptors in the OVCAR-3 cell line was as follows; MUC-1 was strongly expressed, uPA moderately expressed, but HER2 was negative [Song 2006]. 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 4) [29].

The efficacy, biodistribution and long-term toxicity of targeted alpha therapy were investigated for an ovarian ascites mouse model [29]. 213Bi 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 213Bi-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 (Figure 5) [30].

A high tumour: blood ratio (5.8) was found in the biodistribution 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. (Figure 6) [30].

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) [30]. 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.

Conclusion

The collective studies reviewed in this paper show that the MUC1-CE epitope is strongly expressed in prostate, pancreatic and ovarian cancers. Further, Bismuth-213 labelled MAb 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.

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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 labelled with the alpha emitting radioisotope Bismuth-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 I and 2 clinical trials to determine the maximum tolerance dose in humans and the therapeutic efficacy at that dose.

References