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ADVANCES IN RADIOTHERAPY SPECIAL FEATURE: REVIEW ARTICLE

Improving external beam radiotherapy by combination with internal irradiation

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ABSTRACT

The efficacy of external beam radiotherapy (EBRT) is dose dependent, but the dose that can be applied to solid tumour lesions is limited by the sensitivity of the surrounding tissue. The combination of EBRT with systemically applied radioimmunotherapy (RIT) is a promising approach to increase efficacy of radiotherapy. Toxicities of both treatment modalities of this combination of internal and external radiotherapy (CIERT) are not additive, as different organs at risk are in target. However, advantages of both single treatments are combined, for example, precise high dose delivery to the bulk tumour via standard EBRT, which can be increased by addition of RIT, and potential targeting of micrometastases by RIT. Eventually, theragnostic radionuclide pairs can be used to predict uptake of the radiotherapeutic drug prior to and during therapy and find individual patients who may benefit from this treatment. This review aims to highlight the outcome of pre-clinical studies on CIERT and resultant questions for translation into the clinic. Few clinical data are available until now and reasons as well as challenges for clinical implementation are discussed.

External beam radiotherapy (EBRT) alone and in combination with surgery and/or chemotherapy is one of the main modalities for cancer treatment and has a high potential to permanently cure solid tumours even in locally advanced stages by inactivation of cancer stem cells.¹ EBRT can be administered precisely to a target volume during a course of fractionated irradiation. The homogeneous energy dose has a high intensity in solid tumour lesions. For some cancers, survival rates after primary radiotherapy are high [e.g. early stage larynx cancer and early stage non-small-cell lung cancer (NSCLC)], whereas for many other entities they are not (e.g. glioblastomas, sarcomas and advanced NSCLC).²

One way to improve radiotherapy is to increase the inactivation of tumour cells. However, the applicable EBRT dose is limited by the radiosensitivity of the surrounding tissue. While EBRT is directed to the local tumour disease, the use of systemic radioimmunotherapy (RIT) offers the

possibility to treat both, localized and diffuse tumours and (micro)metastases.³ Radionuclides are bound to carrier molecules that target tumour cells. Thus, they are distributed according to the properties of the tracer and are continuously effective during a longer period compared with EBRT, although dose rates decrease depending on the half-life of the radionuclide. Some free therapeutic radionuclides are effective for specific indications, e.g. ¹³¹I for treatment of thyroid cancer or palliative use of ²²³Ra against bone metastases. However, these cannot be translated to treatment of other entities. Besides, radioactive-labelled cytostatic drugs and hormone derivatives,⁴ particularly monoclonal antibodies (mAb) have been radiolabelled and investigated.⁵ Given a substantial difference in the target receptor expression between the tumour cells and surrounding normal tissues, a dose fall-off between both tissues can be expected. The radiolabelled mAb Zevalin® ibritumomab tiuxetan (Zevalin®, Bayer Healthcare Pharmaceuticals, Berlin, Germany) directed

against CD20 is approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of follicular B-cell non-Hodgkin's lymphomas, which are generally considered as radiosensitive. However, mAb are large and are thus taken up slowly into solid tumour tissue followed by a long clearance. Additionally, accumulation in solid tumours depends on vascularization, vessel permeability, tumour size, interstitial pressure and other microenvironmental characteristics.^{6,7} Furthermore, mAb are rather susceptible when labelling under rough conditions. Thus, the application of molecules such as fragment antigen-binding (Fab),^{8–10} nanobodies,^{11,12} affibodies,^{13,14} single chain variable fragments (scFvs),^{15,16} aptamers^{17–19} or peptides^{20,21} is considered. In addition to the effects on target cell, radionuclides with sufficient radiation path length (e.g. β -emitters) can destroy adjacent tumour cells by the crossfire effect, that is through the range of radiation in tissue, cells can be killed without having bound the radionuclide itself.²² This is regarded as a main advantage of RIT for the treatment of solid tumours as plasticity of tumour cells (e.g. loss of target antigen) and delivery barriers can be overcome by some extent. However, the dose-limiting organ in non-myeloablative RIT is the red bone marrow and myelosuppression the main toxicity.²² Therefore, the maximum tolerated activity that was applied in clinical RIT trials (reviewed in Navarro-Teulon *et al*)²³ did not result in tumour doses >33 Gy in large tumours, which is not enough to achieve permanent local control of solid tumours.

Combination of internal and external radiotherapy

The combination of internal (incorporated) and external radiotherapy (CIERT) is a novel promising approach in

radiation oncology. In this review, CIERT is defined more specifically by an integrated (without interval) application of EBRT and systemically applied RIT. Other approaches such as the combination of external radiotherapy with selective internal radiotherapy, radioembolization, brachytherapy, seed implantation, other intravenously applied radionuclide therapies or sequential application of any of these treatments will not be considered here. Furthermore, the focus will be on solid tumours.

The potential benefit of such a combined irradiation is to increase the energy dose applied to the solid tumour lesion, while respecting the limitations of the surrounding normal tissues and the organs at risk (OARs) that are different for both treatment modalities (see above). Figure 1 summarizes the characteristics and OARs of EBRT and RIT and gives an overview on the advantages of the combinatorial approach. Beyond local treatment intensification, another advantage of CIERT can be the combination of local treatment, directed to the solid tumour, and systemic treatment, directed to the subclinically disseminated disease, that is, microscopic tumour lesions not detectable on imaging.

Many challenges are to be met prior to the initiation of CIERT. For example, thoughts on the treatment schedule of CIERT and dosimetry considerations are inevitable. The EBRT would usually be applied as standard treatment. Considerations on the RIT part equal usual aspects of RIT, for example, application of cold doses as well as the choice of the carrier molecule (according to

Figure 1. Combination of internal and external radiotherapy (CIERT). Treatment characteristics of external beam radiotherapy (EBRT) and radioimmunotherapy (RIT) are summarized and advantages of the combination strategy (CIERT) are depicted. Local treatment of the solid tumour via precise EBRT is supplemented by a systemically applied radiotherapeutic drug. Thereby, the tumour dose is enhanced without additional toxicity and (micro)metastasis are potentially targeted. Further, usage of theragnostic radionuclide pairs has the potential to predict delivery and dose distribution of RIT before and during treatment. OAR, organ at risk.

the tumour target) and radionuclides. Accordingly, new developments in the field of RIT, for example, pre-targeting strategies, might be applicable for CIERT approaches in the future but have not been used in this context so far. Many of those aspects are intensively researched with regard to single treatments and reviewed elsewhere.^{3,23–29} This work focuses on the presentation of pre-clinical and clinical investigations on CIERT as a promising treatment strategy.

Choice of radionuclides and theragnostic potential of combination of internal and external radiotherapy
The main factor of radiation toxicity is damage of DNA. If the amount and severity of radiation-induced damage exceeds the repair capacity of the cell, death occurs during mitosis. The linear energy transfer (LET) describes the energy released by the radiation over a certain distance and influences relative biological effectiveness (RBE).^{3,30} X-rays as well as γ - and β -emitters have low LET and thus produce individual DNA lesions mainly by indirect ionization that can easily be repaired. By contrast, high and intermediate LET particle emitters cause clusters of DNA damage that are difficult to repair. Thus, α -emitters (high LET) and Auger electrons (intermediate LET) are more cytotoxic at equivalent absorbed doses. The track path length of α -emitters covers only some cell layers (50–100 μm), and Auger electrons have an even shorter range (<1 μm), which, together with the high RBE, makes them suitable for treatment of small volumes such as micrometastasis.^{3,31} If larger solid tumours are targeted, microenvironmental factors such as perfusion, vessel permeability and the amount of connective tissue influence the distribution of RIT therapeutics. Thus, the application of β -particles may be most promising for CIERT because their path length of 0.5–12.0 mm enables the crossfire effect.^{3,30}

In contrast to mitotic catastrophe caused by irradiation, apoptosis can be induced by some mAb via blockage of the respective receptor and modification of downstream signalling. Thus, the combination of irradiation and mAb may promote the manifestation of sublethal harm to severe damage, which finally lead to cell death. In case of CIERT, radiation is not only applied via EBRT but also by radionuclides bound to the mAb.

A fundamental requisite for the success of radioactivity delivery into solid tumours is that the radionuclide reaches the target and accumulates for an appropriate period. Thus, the pharmacological half-life of the carrier and half-life of the radioactive decay of the chosen nuclide need to be balanced.^{3,23} Most pre-clinical and clinical studies on CIERT used large mAb (approximately 150 kDa), which show a slow plasma clearance. Thus, intratumoral accumulation peaks usually several days after injection. Accordingly, most studies used β -emitters or emitters of Auger electrons with half-lives of at least several days. Pickhard et al.³² recently showed the benefit of using ^{213}Bi bound to an antibody against the epidermal growth factor receptor (EGFR) in combination with EBRT. They demonstrated that different cell death pathways are triggered by this α -emitter and photon irradiation. However, the short half-life of ^{213}Bi (45 min) may limit its usage for solid tumours *in vivo* if the nuclide is

linked to antibodies, because most doses will be applied before the tracer penetrates into tumour tissue. Thus, ^{213}Bi may only be useful to treat haematological malignancies and therefore is not feasible for CIERT. The concept of pre-targeting is intensively researched in association with RIT as a single treatment. The tumour is pre-targeted with the unlabelled complementary prepared antibody, and the radionuclide is delivered via a small molecule recognizing the antibody by the complementary system in a second step. This may lead to higher tumour uptake with lower normal tissue retention (reviewed in van de Watering et al.²⁹). However, a combination with EBRT has never been investigated and substantial research on scheduling would be mandatory.

The concept of theragnostic approaches is applicable for CIERT, as theragnostic radionuclide pairs can be used for the RIT part of the therapy. The goal is to combine a diagnostic tool having an imaging radionuclide (positron or γ -radiation emitter) with a derived individualized therapeutic procedure using a therapeutic radionuclide (particle emitter). The tumour and normal tissue uptake of the respective drug can be evaluated for individual patients via positron emission tomography (PET) or single photon emission CT (SPECT) and give predictive information on a potential treatment benefit. The selection of appropriate radionuclides for imaging with regard to their replacement by a radionuclide for therapeutic purposes that exhibit similar chemical and physical properties is a crucial matter. Thus, it is important to consider different characteristics of radiation according to the requirements, such as decay characteristics, dose range and physical half-life of the radionuclides.³⁰ Imaging with radionuclide-labelled conjugates provides pre-therapeutic information such as biodistribution, hints of a limiting or critical organ or tissue, and maximum tolerated dose. Dosimetry is most challenging, as pre-therapeutic imaging may not be congruent to actual delivered doses.³³ However, this field is extensively investigated for peptide receptor radionuclide therapy (PRRT), and results are directly transferable to CIERT approaches. After applying therapeutic nuclide-labelled conjugates, the results of such treatment may again be monitored via imaging. A selection of theragnostic combinations of radionuclides are shown in Table 1. ^{64}Cu is denoted here as PET radionuclide but with a considerable part of β^- -radiation as well as low-energy Auger electrons (0.6–8.3 keV), it has the potential for radiotherapeutic application. However, the half-life is rather short. Furthermore, ^{67}Cu releases γ -radiation directly following the β^- -decay; thus, it can be used for SPECT,⁵¹ but its production is difficult and expensive.⁵² The positron emitters ^{86}Y and ^{124}I have been described controversially as PET nuclides since besides high β^+ -radiation energy they emit multiple high-energy γ photons that cause so-called multiple coincidences disturbing PET imaging quality. However, different correction methods allow improved quantitative imaging.⁵⁰ Moreover, for the application of ^{90}Y -labelled radiopharmaceuticals, it is suggested to estimate the uptake and dosimetry with the nuclide counterpart ^{86}Y .³⁶ Nevertheless, ^{86}Y -PET is far from clinical routine, at least in the near future. Furthermore, ^{131}I also emits γ -radiation that has been used for imaging, and ^{111}In and ^{123}I have a potential for treatment owing to their released Auger electrons.

Table 1. Potential theragnostic radionuclides^a

Pair	Half-life	Radiation (keV)	Application examples			
			Study	Imaging	Model	Entity
⁶⁴ Cu/ ⁶⁷ Cu	12.7 h/ 2.6 days	β^+ 653 (17.5%)/ β^- 562 (100%) ^b	Anderson and Ferdani ³⁴ / Novak-Hofer and Schubiger ³⁵	PET; small animal PET/SPECT; biodistribution	Patients hypoxia; mice (tm) mAb/ patients mAb; mice mAb Fabs	lc, cc; SCC/NHL, colc, bc; nb, colc
⁸⁶ Y/ ⁹⁰ Y	14.7 h/ 2 days	β^+ 2766 (17.5%) ^c / β^- 2280 (100%)	Lopci et al ³⁶ / McKinney and Beaven ³⁷	Small animal PET	mice (tm) mAb/ patients mAb (Zevalin®)	Different xenografts/NHL
⁸⁹ Zr/ ⁹⁰ Y	3.3 days/ 2.7 days	β^+ 902 (22.7%) ^c / β^- 2280 (100%)	Osborne et al ³⁸ / Perk et al ³⁹	PET; biodistribution	Patients mAb/ patient mice (tm) mAb (Zevalin)	pc/NHL
⁸⁶ Y/ ¹⁷⁷ Lu	14.7 h/ 6.6 days	β^+ 2766 (17.5%) ^c / β^- 498 (79%) A.e. 4.3–65.3	Lopci et al ³⁶ / Liu et al ⁴⁰	Small animal PET/ small animal SPECT	mice (tm) mAb/ mice (tm) mAb	Different xenografts/ HNSCC
⁸⁹ Zr/ ¹⁷⁷ Lu	3.3 days/ 6.6 days	β^+ 902 (22.7%) ^c / β^- 498 (79%) A.e. 4.3–65.3	Osborne et al ³⁸	PET	Patients mAb	pc
^{99m} Tc/ ¹⁸⁶ Re	6 h/ 3.7 days	γ 140 (99%)/ β^- 1069 (71%) A.e. 4.5–69.5	Nagar et al ⁴¹	SPECT	Patients MIBI	Parathyroid adenoma
^{99m} Tc/ ¹⁸⁸ Re	6 h/17 h	γ 3140 (99%)/ β^- 1069 (71%) A.e. 47.7–69.9	Müller et al ⁴²	Biodistribution	mice (tm) folate	nasc
¹¹¹ In/ ⁹⁰ Y	2.8 days/ 2.7 days	γ 171; 245 (100%)/ β^- 2280 (100%)	O'Donnell et al ⁴³	SPECT	Patients mAb	pc
¹²³ I/ ¹³¹ I	13.2 h/ 8 days	γ 159 (97%)/ β^- 606 (89%)	Bravo et al ⁴⁴	SPECT	Patients NaI	thc
¹²⁴ I/ ¹³¹ I	4.2 days/ 8 days	β^+ 3673 (23%) ^c / β^- 606 (89%)	Van Nostrand et al ⁴⁵	PET	Patients NaI	thc
¹²⁴ I/ ¹⁸⁶ Re	4.2 days/ 3.7 days	β^+ 3673 (23%) ^c / β^- 1069 (71%) A.e. 4.5–69.5	Verel et al ⁴⁶	Biodistribution	mice (tm) mAb	HNSCC
¹²⁴ I/ ¹⁸⁸ Re	4.2 days/ 17 h	β^+ 3673 (23%) ^c / β^- 2120 (71%) A.e. 47.7–69.9	Verel et al ⁴⁶ /Torres et al ⁴⁷	Biodistribution/ SPECT	mice (tm) mAb/ patients mAb	HNSCC/glioma

A.e, Auger electrons; bc, bladder cancer; cc, cervical carcinoma; colc, colon carcinoma; Fab, Fragment antigen binding; HNSCC, head and neck squamous cell carcinoma; lc, lung carcinoma; mAb, monoclonal antibodies; MIBI, methoxy isobutyl isonitrile; nasc, nasopharyngeal carcinoma; nb, neuroblastoma; NHL, non-Hodgkin's lymphoma; pc, prostate cancer; PET, positron emission tomography; SCC, squamous cell carcinoma (A431); SPECT, single photon emission CT; thc, thyroid cancer; tm, tumour model.

^aData from Laboratoire National Henri Becquerel: http://www.nucleide.org/DDEP_WG/DDEPdata.htm.⁴⁸

^b<http://periodictable.com/Isotopes/029.67/index3.p.full.dm.prod.html>.⁴⁹

^cData from Lubberink and Herzog.⁵⁰

PRE-CLINICAL RESULTS: PROMISES AND CHALLENGES OF COMBINATION OF INTERNAL AND EXTERNAL RADIOTHERAPY

Pre-clinical investigations on CIERT have been published for more than 25 years^{53,54} and are summarized in Table 2. Comparison of different data sets is limited because they differ in almost all aspects: model systems, tumour entities, target antigens, radionuclides, delivered activities and doses, treatment schedules and end points. Pre-clinical investigations are used to gain knowledge on principal radiobiological aspects, associations of treatment schedules, target antigen and tumour entity with outcome as well as on principal efficacy.

Pre-clinical model systems and their impact on combination of internal and external radiotherapy research

Selection of the model system for pre-clinical experiments on CIERT is of high relevance and should be decided in light of the research question addressed. Four of the studies summarized in Table 2 were investigated in a German framework for radiation research. Herein, the same therapeutic antibody [cetuximab (Erbix®; Merck KGaA, Darmstadt, Germany) labelled with ⁹⁰Y], and the same panel of head and neck squamous cell carcinoma (HNSCC) cell lines were used. This offers the possibility

Table 2. Pre-clinical studies on combination of internal and external radiotherapy

Model	Entity	Nuclide	mAb	Therapy schedule ^a	EBRT dose	RIT dose	End point	Study
Two dimensional cell cultures	Glioblastoma, colorectal	¹²⁵ I	Murine anti-EGFR (mAb 425)	EBRT, after 2 days RIT	6 Gy	0.37 MBq ml ⁻¹	Cell growth	Bender et al ⁵⁵
	Squamous carcinoma of vulva	¹³¹ I	Humanized murine anti-EGFR (Cetuximab)	Imprecise, probably simultaneous	2, 4, 10 Gy	0.1 MBq ml ⁻¹	Cell growth	Rades et al ⁵⁶
	HNSCC, squamous carcinoma of vulva	⁹⁰ Y	Humanized murine anti-EGFR (Cetuximab)	Simultaneous	2, 4 Gy	1 MBq	Clonogenicity	Saki et al ⁵⁷
	HNSCC, squamous carcinoma of vulva	⁹⁰ Y	Humanized murine anti-EGFR (Cetuximab)	EBRT, after 4 h RIT	2, 4, 6 Gy	0.5, 1, 1.5 MBq ml ⁻¹	Clonogenicity	Eke et al ⁵⁸
	HNSCC	²¹³ Bi	Humanized murine anti-EGFR (Matuzumab)	Simultaneous	2 Gy	0.037 MBq ml ⁻¹	Clonogenicity	Pickhard et al ³²
Three dimensional spheroids	HNSCC	⁹⁰ Y	Humanized murine anti-EGFR (Cetuximab)	Simultaneous	2.5–25 Gy	1.2 MBq ml ⁻¹	Spheroid control	Ingargiola et al ⁵⁹
Xenograft tumours in mice	Breast	¹¹¹ In	Murine anti-γH2AX with TAT motive	RIT, after 1 h EBRT	10 Gy	60 MBq	Clonogenicity, tumour growth delay	Cornelissen et al ⁶⁰
	Breast, HNSCC	¹¹¹ In	Murine anti-γH2AX with NLS and EGFR-targeting properties	RIT, after 1 h EBRT	10 Gy	5 MBq	Clonogenicity	Cornelissen et al ⁶¹
	Colorectal	¹³¹ I	3 F(ab') ₂ of murine anti-CEA (mAb 35, CE25-B7, B93)	fx EBRT, on last day fx RIT starts	16–32 Gy in 5 fx	2 × 55.5 MBq	Tumour growth delay, tumour control	Buchegger et al ⁶²
	Colorectal	¹³¹ I	4 murine anti-CEA (mAb 35, B7, B93, B17)	Various	30, 40 Gy in 10 fx	3 × 7.4 MBq	Tumour growth delay	Sun et al ⁶³
	Cervical adenocarcinoma	¹³¹ I	Murine anti-PLAP + anti-CK8 (H7, TS1)	3 fx EBRT, after 1 week RIT	15 Gy in 3 fx	20 MBq	Tumour growth delay	Eriksson et al ⁶⁴
	HNSCC	⁹⁰ Y	Humanized murine anti-EGFR (Cetuximab)	EBRT, after 2–4 days RIT	14–50 Gy	2.8 MBq	Tumour control	Koi et al ⁶⁵
Liver metastasis in mice	Colorectal	¹³¹ I	4 murine anti-CEA (mAb 35, CE25-B7, B93, B17)	5 fx EBRT, RIT, 5 fx EBRT, RIT	20 Gy in 10 fx	2 × 5.55 MBq	Survival	Vogel et al ⁶⁶

γH2AX, phosphorylated histone H2AX; CEA, carcinoembryonic antigen; CK8, cytokeratin 8; EBRT, external beam radiotherapy; EGFR, epidermal growth factor receptor; fx, fractionated; HNSCC, head and neck squamous cell carcinoma; mAb, monoclonal antibodies; NLS, nuclear localization sequence; PLAP, placental alkaline phosphatase; RIT, radioimmunotherapy; TAT, peptide of the trans-activator of transcription protein of HIV-1 virus.

^aIf treatments directly followed each other this was counted as simultaneously.

to compare the results obtained in different model systems along the translational path from two-dimensional (2D) cell cultures to pre-clinical *in vivo* models. Within this consortium, Saki et al⁵⁷ demonstrated a decrease of the surviving fraction of the HNSCC cell line UT-SCC-5 after combination of EBRT with cold Cetuximab, which was further reduced by CIERT. However, this cell line was a non-responder in a study of Gurtner et al⁶⁷ who combined EBRT with (cold) Cetuximab treatment in xenograft tumours. Koi et al⁶⁵ showed 2 years later that UT-SCC-5 also did not respond to CIERT when compared with EBRT alone in a tumour control analysis in xenografts. This discrepancy between 2D cell cultures and *in vivo* results is likely caused by the pharmacokinetics of the therapeutic antibody *in vivo*. Indeed, UT-SCC-5 xenograft tumours were shown to be the least perfused tumour of a panel of six HNSCC models.⁶⁸ Furthermore, a histological evaluation and PET study in Koi et al⁶⁵ demonstrated the lowest tumour uptake and an uneven distribution of the radiolabelled antibody for UT-SCC-5 xenografts compared with two other models. In the same study, a substantial reduction of the tumour control dose 50 (TCD50, dose of EBRT that cures 50% of the xenograft tumours) with CIERT was demonstrated for FaDu xenografts.⁶⁵ Of note, tumours of this cell line were well perfused and showed the highest uptake of Cetuximab in the PET analysis.^{65,68} However, they did not or only marginally responded to the combination of EBRT and cold Cetuximab,^{65,67,69} which leads to the conclusion that the antibody functioned as a carrier of the radionuclide in the CIERT study but did not contribute by itself to the therapeutic effect. The result demonstrates the promising potential of CIERT if the radiolabelled agent accumulates in the target tumour and is well distributed. This encourages the development of theragnostic approaches that have the potential to identify patients who will benefit from CIERT.^{5,65}

Ingargiola et al⁵⁹ adopted three-dimensional (3D) spheroid cultures to the *in vivo* tumour control assay. This approach reflects the 3D morphology of tumour cells and pathophysiological gradients (oxygen, metabolites, pH), which may influence their intrinsic radiosensitivity.^{70,71} CIERT compared with EBRT alone led to a pronounced enhancement of spheroid control probability in the SAS cells as a model. However, SAS and FaDu were already controlled after RIT treatment alone in spheroids as well as 2D cultures.^{58,59} The obviously higher RIT efficacy *in vitro* than in xenografts may be explained by differing RIT doses used in the studies. Regardless of the limitations of efficacy tests, *in vitro* cell culture models offer important possibilities to study other end points such as cellular binding and uptake^{55,57,59} as well as downstream signalling.^{32,57,58} Accordingly, *in vitro* models should be used to uncover underlying mechanisms and can give indications on efficacy. However, to evaluate the therapeutic benefit of combined treatment strategies, *in vivo* studies are inevitable for translation into the clinic. In general, only a minority of investigators used tumour control as end point.^{62,63,65} However, studying curative end points in preclinical trials is a pre-requisite for translation into clinical protocols with curative aim as promising results for tumour growth delay not always lead to enhanced control probability.^{67,72} Additionally, the investigation of normal tissue toxicity should be performed in CIERT studies, as

sparing of normal tissue dose is a major aspect of this treatment strategy. Again, only few studies paid attention to this complex issue, which may require the investigation of orthotopic tumour models. Moreover, if specific human antigens are targeted, animal models with transplanted human tumours fail to predict normal tissue response, as the target is not present on murine normal cells.

Scheduling of combination of internal and external radiotherapy

A crucial question that should be addressed in pre-clinical experiments on CIERT is the treatment schedule. Standard EBRT in cancer treatment is applied in multiple daily fractions over several weeks to enable normal tissue repair. Hence, radiolabelled antibodies could be injected before, during or after finishing radiation or can be applied also fractionated, which again offers innumerable possibilities for combination.

In the *in vivo* studies included in Table 2, a wide variety of schedules were applied. Many early studies showed enhanced tumour uptake of radiolabelled antibodies after EBRT.^{53,54,73,74} Suggested reasons were an increase of the vascular permeability after EBRT⁷³ and the shrinkage of tumour mass^{54,75} with RIT being more effective on smaller target volumes.^{66,75} The effect was dose dependent and did not lead to a higher uptake in normal tissues if X-rays were used.^{53,63,74} Interestingly, Gridley et al⁷⁴ showed that the tumour uptake of an anti-carcinoembryonic antigen (CEA) antibody could even be increased by using protons for EBRT instead of X-rays. However, this was accompanied by higher accumulation in normal tissue and traced back to a stronger antigen secretion after proton irradiation. These early studies focused on the improvement of RIT, and tumour uptake was the main end point measured.

The first systematic pre-clinical study on CIERT as a curative combined approach was investigated in 1995 by Buchegger et al.⁶² A fractionated schedule and high doses of EBRT were combined with high injected RIT activities [mixture of different anti-CEA F(ab')₂ fragments]. Of significance, not only tumour uptake was analysed but also efficacy of treatment. The results show a prominent advantage of CIERT when compared with the single treatments in two tumour models without additional toxicity. The same group investigated if tuning of treatment schedule may further increase the outcome.⁶³ Simultaneous application of EBRT and RIT, both in a fractionated scheme, turned out to be most effective causing the longest growth delay compared with other treatment timings in this trial. The associated biodistribution experiment revealed no pronounced changes of antibody uptake in tumours irradiated with low doses but a reduced uptake if 30 Gy (10 fractions) were applied prior to the antibody injection. Additionally, a clear disadvantage of a long delay between EBRT and RIT was shown. However, uptake was not investigated for the complex fractionated timing with the highest therapeutic potential. This schedule was further tested on orthotopic liver metastases of a lung tumour model.⁶⁶ Again, a benefit of CIERT over the single treatments was demonstrated, which led to translation of the approach into a clinical feasibility study.⁷⁶

Ruan et al⁷⁵ addressed the fact that the efficacy of RIT and the tumour response are influenced by the uniformity of antibody microdistribution in the target tissue. To this end, the authors investigated tumour uptake as well as distribution of a radiolabelled antibody directed against the surface protein A33 on autoradiographic slices for different treatment schemes. The simultaneous application of EBRT and RIT led to the highest uptake, but the radiolabelled antibodies were most evenly distributed in non-irradiated tumours. However, no efficacy analysis of the different treatment schedules was performed in this study, making conclusions on the impact of the radionuclide uptake and distribution on treatment response speculative. Generally, the impact of uneven distribution on treatment success strongly depends on the crossfire effect. The magnitude of this effect relies on the used radionuclide and the tissue penetration depth of emitted radiation and may overcome poor dissemination of the radiotherapeutic drug.^{3,22}

Taken together, the application of RIT simultaneously with or after EBRT was in most cases beneficial to a schedule where RIT was applied prior to external irradiation. It may not make a difference if fractionated EBRT starts some days prior to RIT injection or on the same day, as mAb circulate for several days. However, there are indications that the effect of increased antibody uptake after EBRT disappears at higher doses.^{62,63} The optimal treatment schedule has to be defined for each individual antigen/antibody pair and may be influenced by the chosen tumour model as well. Additionally, increased connective tissue amounts and cystic composition as well as low numbers of residual viable tumour cells were observed in xenografts treated with CIERT compared with single treatments. Thus, not only increased dose delivery but also other radiobiological processes may be involved in the promising pre-clinical results obtained with CIERT. To this end, further pre-clinical research is required to understand mechanisms and enhance the potential of this promising combined treatment approach.

Noteworthy, all studies involving efficacy testing show a benefit of CIERT over single treatments, and normal tissue was spared if this was investigated, as far as this end point can be judged in mouse models. The addition of other approaches to enhance uptake or specificity may further improve CIERT. For example, Cornelissen et al⁶⁰ were able to generate DNA double strand breaks (dsb) in tumour cells via EBRT, which were subsequently targeted by RIT via an antibody construct with a nuclear targeting sequence, which was directed against the dsb-binding histone γ H2AX. In fact, they could find a massive benefit on tumour growth delay of this CIERT strategy in comparison with single treatments. Subsequently, the construct was further extended by EGFR-targeting properties to avoid uptake in normal cells situated in the radiation field.⁶¹ However, this strategy may be unfavourable if metastatic disease is a target, because only cells in the radiation field increase γ H2AX after irradiation. Nevertheless, the elegant format of the construct shows how intracellular antigens can be targeted. Furthermore, a high specificity was mediated by the combined targeting of a surface receptor and an induced intracellular antigen.

TRANSLATION OF COMBINATION OF INTERNAL AND EXTERNAL RADIOTHERAPY INTO CLINICAL STUDIES

CIERT is composed of EBRT as a local therapy and RIT as a systemic treatment. This approach strictly requires interdisciplinary teamwork between nuclear medicine and radio-oncology.

External radiotherapy is much better understood and therefore widely used in the majority of oncological treatment options, whereas nuclear medicine treatments, except radioiodine treatment of thyroid cancer, are relatively rare and their mechanisms of action are much less known. But beyond radioiodine, these treatments further include RIT of lymphoma, PRRT (e.g. with somatostatin receptor analogues) or systemic treatment of bone metastases with bone-seeking radiotracers [e.g. $^{223}\text{Ra-Cl}_2$ or $^{153}\text{Sm-ethylenediamine tetra (methylene phosphonic acid) (EDTMP)}$]. Nevertheless, mAb and their fragments are the most popular experimental carriers for radiolabelling. Therefore, first approaches of diagnostic and/or therapeutic applications have been reported since their first synthesis in the early 1980s.⁷⁷ Consequently, the base of successful *in vivo* tumour targeting after intravenous application were antigen–mAb binding (e.g. antiferritin mAb),⁷⁷ the use of the avidin–biotin binding⁷⁸ and mAb binding to receptors, mostly EGFR.⁷⁹ Not surprising, the radioisotopes, e.g. ^{90}Y , ^{131}I and ^{125}I , ^{111}In (for dosimetry), applied for the systemic part of CIERT are still in use today.^{77,80,81} With regard to the literature, reports about integrated CIERT of solid tumours in males are rare, but several clinical trials addressing different tumour-targeting mechanisms have been conducted for three decades.⁸⁰ The most treated entities with predominantly promising reports are (recurrent) malignant glioma, meningioma, primary and secondary tumours of the liver, brain metastases (especially from NSCLC), osteoplastic bone metastases from prostate cancer, primary breast cancer, malignant pheochromocytoma and paraganglioma. Table 3 gives an overview on early clinical trials. In agreement with pre-clinical results, Order et al⁷⁷ observed an increasing radiotracer uptake after percutaneous radiotherapy followed by intravenous applied systemic radiotherapy. This schedule led to higher energy doses in pre-irradiated tumour regions driven by the inflammatory process initiated by the percutaneous radiotherapy. They achieved imposing remissions but only in rare cases. However, only few studies conducted a real combination of EBRT and RIT; in most cases, a sequential application of the treatment modalities was performed. Furthermore, by the nature of first-in-men trials, the majority of these studies were performed in patients suffering from advanced tumours with a very bad prognosis.⁸¹ The vast majority were carried out as Phase I/II trials to demonstrate safety, feasibility and efficacy. Only four studies were designed as prospective randomized controlled trials. First of all, Order et al⁹¹ conducted a Radiation Therapy Oncology Group study comparing full-dose chemotherapy to cyclic, systemic application of ^{131}I -antiferritin after induction therapy consisting of EBRT integrated with doxorubicin and 5-fluorouracil in patients with unresectable hepatocellular carcinoma. Baczyk et al⁸⁹ compared the combination of intravenously applied $^{153}\text{Sm-EDTMP}$ and percutaneous radiotherapy

Table 3. Clinical combination of internal and external radiotherapy trials

Study	Patients	Entity	Radiotracer	Intention	Mode
Msirikale <i>et al</i> ⁵³	105	Hepatoma	¹³¹ I-antiferritin mAb	Phase I/II	Sequential
Msirikale <i>et al</i> ⁵³	6	Hepatocellular cancer	⁹⁰ Y-antiferritin mAb	Phase I	Sequential
Msirikale <i>et al</i> ⁵³	48	Hepatocellular cancer	¹³¹ I-antiferritin mAb	Phase III comparison with chemotherapy	Sequential
Porter <i>et al</i> ⁸²	126	Bone metastases (PCA)	⁸⁹ Sr-Cl ₂	Phase III, comparison with mono EBRT	Combined
Buchegger <i>et al</i> ⁷⁶	6	Liver metastases (colorectal cancer)	¹³¹ I-mAb F(ab') ₂	Feasibility	Combined
Quang and Brady ⁸³	180	Malignant glioma	¹²⁵ I-anti-epidermal growth factor receptor-mAb 425	Phase II	Sequential
Nilsson <i>et al</i> ⁸⁴	64	Bone metastases (PCA)	²²³ Ra-Cl ₂	Phase II, comparison with mono EBRT	Combined
Li <i>et al</i> ⁷⁹	192	Malignant glioma	¹²⁵ I-mAb	Phase II	Sequential
Rades <i>et al</i> ⁵⁶	1	Brain metastases (non-small-cell lung cancer)	¹²⁵ I-Cetuximab	Feasibility	Sequential
Burdick <i>et al</i> ⁸⁵	11	Follicular lymphoma	⁹⁰ Y-ibritumomab tiuxetan	Feasibility	Sequential
Hobbs <i>et al</i> ⁸⁶	1	Paraspinal tumour	¹⁵³ Sm-EDTMP	Treatment planning	Sequential
Ferrari <i>et al</i> ⁷⁸	14	Breast cancer	⁹⁰ Y-biotin	Feasibility	Sequential
Fishbein <i>et al</i> ⁸⁷	5	Paraganglioma	¹³¹ I-metaiodobenzylguanidine	Feasibility	Sequential
Kreissl <i>et al</i> ⁸⁸	10	Meningioma	¹⁷⁷ Lu-DOTA conjugates	Feasibility	Combined
Baczyk <i>et al</i> ⁸⁹	88	Bone metastases (PCA)	¹⁵³ Sm-EDTMP	Phase III, comparison with monotherapy	Combined
Verburg <i>et al</i> ⁹⁰	5	Malignant glioma	¹³¹ I-phenylalanine	Feasibility	Sequential

DOTA, 1,4,7,10-tetra-azacyclododecane-1,4,7,10-tetra-acetic acid; EBRT, external beam radiotherapy; EDTMP, ethylenediamine tetra(methylene phosphonic acid); mAb, monoclonal antibodies; PCA, prostate cancer.

with ¹⁵³Sm-EDTMP alone in bone metastases. Furthermore, Porter *et al*⁸² and Nilsson *et al*⁸⁴ conducted similarly designed studies using the radionuclides ⁸⁹Sr and ²²³Ra; noteworthy, the latter one is actually a FDA- and EMA-approved α -emitter. However, treatments for bone metastases always do have a palliative intention,⁸⁹ thus allowing no conclusion on any improvement of tumour curability. Nevertheless, all four authors could conclude the superiority of CIERT to the monotherapy.

Open questions for clinical application of combination of internal and external radiotherapy
Alongside the acquisition of knowledge in basic radiobiological mechanisms, the biggest “clinical” step forward was made in the field of 3D dosimetry. This is reflected by a significant number of outstanding publications over the past decade accomplishing both radio-oncological standards as well as those of nuclear medicine.^{78,92–96} Nevertheless, despite more or less efficient teamwork between those in nuclear medicine and the radio-oncologists, dosimetry of an internal irradiation respecting all the important factors as residence time of mAb or its fragments,

individual differences in individual patient’s organ functions, radiation quality (β -emitter) and inhomogeneity of dose prescription is still challenging. Is a radio-oncological “gray” really similar to that in nuclear medicine? That is the question here, and confidence, despite good practice and scientific knowledge, is required too. Considerably fewer articles were published addressing temporal relationships and design considerations of CIERT.^{24,76,97,98} Another important question is the order of the combined treatments. A further, still controversially discussed question is the manner of antibody application. Whereas Quang and Brady⁸³ applied “cold”, unlabelled antibody at first, followed by the “hot”, labelled after few days, Rades *et al*⁹⁹ conducted an inverse sequence. It is well known from the FDA- and EMA-approved RIT with ibritumomab tiuxetan (Zevalin) that pre-conditioning with the “cold” antibody can have distinct advantages, but this is depending on the individual mAb, which may be the biggest challenge of CIERT in the near future. Specifically for mAb, the distribution in small animals is different from the one in humans (*e.g.* EGFR-mAb and CEA-mAb), limiting conclusions especially on normal tissue toxicities. Chimeric or

humanized mouse models are used today for pre-clinical drug development. For example, liver-humanized mice show similarities to the human liver in drug metabolism and toxicity, which are not reflected in normal mice owing to differences in metabolic enzymes and transporters.^{100,101} However, genetically engineered mouse models that express the desired human antigen in the same cell types as humans have only been created for lymphoma models so far.¹⁰² Mice with humanized-receptor expression in normal tissues would have the potential to better reflect the distribution and toxicity of mAb variants and could also be used to study pharmacodynamics of radiolabelled constructs. However, dosimetric images do not allow discrimination between unspecific uptake of mAb in the human liver from specific binding on the receptor addressed.

Taken together, several issues of CIERT are solved, for instance mAb labelling and imaging, or—at least partly—3D dosimetry of intravenously applied radiotracers. The main effort for future research into this treatment option will be the acquisition of knowledge about specific distributions of mAb-based radiotracer in humans, and, much more important, to evaluate possibilities to influence and to steer it.

CONCLUSION

The advantages of a combination of EBRT and RIT are prominent. The possibility to use theragnostic radionuclide pairs facilitates individualization of this treatment strategy. Although, the view to a 30-year-long history of CIERT determines the question: why were the improvements of CIERT in three decades so few? The reasons are sophisticated. One reason may be the very high legal and administrative hurdles, at least in some countries, where such developments could be translated to the

clinics. The principal scientific reason, despite more or less efficient teamwork between those in nuclear medicine and the radio-oncologists, is the complexity of a dosimetry of the internal irradiation respecting all the important factors as residence time of carrier molecules, individual differences in patient's organ functions, radiation quality and inhomogeneity of dose prescription. However, knowledge gained by research on the use of (radiolabelled) mAb can be directly transferred to CIERT. Plenty of different strategies to bypass barriers and to enhance tumour uptake are under investigation²³ and can potentially be adapted to combined approaches. From *in vivo* experiments, there is great evidence that the usage of smaller antibody fragments and alternative carrier molecules (aptamers, peptides) may overcome the problem of poor tumour uptake. *In vitro* and animal experiments are inevitable to study principal efficacy of CIERT approaches and to evaluate radiobiological processes. However, the prediction for patient treatment is limited owing to the human(ized) origin of mAb, resulting in a different biodistribution in the animal. Overall, it is an obligate pre-condition for CIERT that classical radiobiology needs to be adapted to the use of open radionuclides for a real comparison with external radiotherapy, an approach that needs to be driven by radio-oncologists together with researchers in nuclear medicine.

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