

## Auger radiation targeted into DNA: a therapy perspective

Franz Buchegger<sup>1,2</sup>, Florence Perillo-Adamer<sup>1</sup>, Yves M. Dupertuis<sup>3</sup>, Angelika Bischof Delaloye<sup>1</sup>

<sup>1</sup> Service of Nuclear Medicine, University Hospital of Lausanne CHUV, Rue du Bugnon 46, 1011 Lausanne, Switzerland

<sup>2</sup> Service of Nuclear Medicine, University Hospital of Lausanne, Lausanne, Switzerland

<sup>3</sup> Service of Nutrition, University Hospital of Geneva, Geneva, Switzerland

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**Abstract.** *Background:* Auger electron emitters that can be targeted into DNA of tumour cells represent an attractive systemic radiation therapy goal. In the situation of DNA-associated decay, the high linear energy transfer (LET) of Auger electrons gives a high relative biological efficacy similar to that of  $\alpha$  particles. In contrast to  $\alpha$  radiation, however, Auger radiation is of low toxicity when decaying outside the cell nucleus, as in cytoplasm or outside cells during blood transport. The challenge for such therapies is the requirement to target a high percentage of all cancer cells. An overview of Auger radiation therapy approaches of the past decade shows several research directions and various targeting vehicles. The latter include hormones, peptides, halogenated nucleotides, oligonucleotides and internalising antibodies.

*Discussion:* Here, we will discuss the basic principles of Auger electron therapy as compared with vector-guided  $\alpha$  and  $\beta$  radiation. We also review some radioprotection issues and briefly present the main advantages and disadvantages of the different targeting modalities that are under investigation.

**Keywords:** Auger radiation – Nuclear targeting – Treatment – Dosimetry

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### Introduction

Current clinically useful systemic radiation therapies are mainly based on  $\beta^-$  radiation emitters [1]. Radioisotopes

such as  $^{131}\text{I}$ ,  $^{32}\text{P}$  and  $^{89}\text{Sr}$  are efficient therapeutic agents because they are taken up by cancerous or hyperactive tissues [2–8]. These  $\beta^-$  emitters and others such as  $^{90}\text{Y}$  and  $^{177}\text{Lu}$  have been coupled to antibodies [9–12] or peptides [13, 14] in order to target them into tumours for systemic radiation therapy. In recent years,  $\alpha$  emitters have regained popularity with the emergence of new production processes and the availability of clinically useful targeting agents such as humanised, tumour-selective monoclonal antibodies. However, studies with  $\alpha$  emitters remain mostly pre-clinical [15, 16]. Similarly, Auger radiation therapy, while being a long-standing research goal, has encountered multiple obstacles, and clinical studies have been very scarce.

Among the different requirements of systemic radiation therapy that we shall discuss below, we first mention the importance of suitable tumour-selective vehicles. Antibodies in lymphoma therapy and peptides that bind to somatostatin receptors are the most advanced options in this respect.

A second requirement, in our opinion, aimed at increasing the efficacy of systemic radiation therapy, is the repeated application of internal emitters. Even though radiolabelled antibodies, as an example, circulate and irradiate tumours over a period of a few days, repetition of therapy might still be favourable. Repetition of therapy cycles is already in use in radio-peptide treatment. Repetition of treatment or, alternatively, continued infusion appears essential with certain radio-nucleotides that can only be incorporated in the S phase of the cell cycle, such as iododeoxyuridine. The aim of repeating radiotherapy cycles is the successive destruction of the most accessible and radiosensitive parts of cancer nodules. The sequential radiotherapy cycles would hit re-oxygenated, re-nourished cycling cells in radiosensitive phases, as is the case in conventional external beam radiation therapy [17, 18].

A third requirement of systemic radiation therapy is the targeting of radiation to large proportions of all live cancerous cells. With respect to large solid tumour nodules, the crossfire effect of  $\beta^-$  particle emitters can be a major advantage [9–12]. The long range tissue penetration, however, might be a disadvantage when targeting small clusters of tumour cells.

Franz Buchegger (✉)  
Service of Nuclear Medicine,  
University Hospital of Lausanne,  
Lausanne, Switzerland  
e-mail: Franz.Buchegger@chuv.ch  
Tel.: +41-21-3144373; Fax: +41-21-3144349

Owing to their physical properties,  $\alpha$  and Auger electron emitters are better adapted for the treatment of individual cells or small clusters. These two radiations provide a high linear energy transfer (high LET) type of energy deposition:  $\alpha$  particles with about 80–100 keV/ $\mu\text{m}$  and Auger electrons with 4–26 keV/ $\mu\text{m}$  [19]. As a consequence,  $\alpha$  radiation has a path length in tissue in the range of only 40–80  $\mu\text{m}$  while the path length of most Auger electrons is well below 1  $\mu\text{m}$ . These radiations are therefore optimal for treatment of individual cancer cells or small cell clusters. The limited tissue penetration of only a few cell diameters and the rather short half-life of most available  $\alpha$  emitters are the major reasons why this type of radiation is mainly appropriate for haematological malignancies [19].

In contrast to  $\alpha$  and  $\beta^-$  radiation, treatment based on Auger electron emitters requires targeting of the radioisotope into individual tumour cells and even into the nucleus. Optimal Auger radiation efficacy is obtained when Auger emitters are tightly bound to DNA. Despite the multiple obstacles that have been encountered, Auger radiation therapy approaches remain appealing because of the selective toxicity for cells that incorporate the radiopharmaceutical into the nucleus. In contrast to  $\alpha$  and  $\beta^-$  radiation, Auger radiation emitters remain of low toxicity while travelling in blood or bone marrow but become highly efficient when incorporated into DNA of target cells.

The high LET type energy deposition of  $\alpha$  and Auger radiation in DNA produces a high proportion of double strand breaks. The double strand destruction of several nucleotides can make DNA repair [20, 21] rather inefficient and prone to error. The relative biological efficacy (RBE) of  $\alpha$  and Auger radiation emitters is thus significantly higher than that of  $\beta^-$  radiation. However, this is the case for both tumour and normal tissues.

### Fundamental requirements of systemic radiation therapy

The first requirement of systemic radiation therapy is the definition of suitable tumour-selective vehicles in order to avoid normal tissue toxicity. Antibodies and peptides, notably those binding to somatostatin receptors, [22], are most advanced in this respect. Furthermore, two anti-CD20 antibodies for radioimmunotherapy (RIT) of lymphoma are currently FDA approved [23, 24].

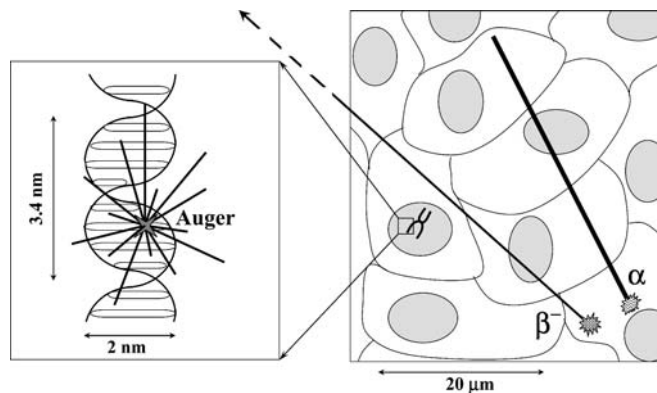
It appears from these therapies that the specificity of antibodies or peptides for tumour is not an absolute requirement. Targeting of normal B cells with anti-CD20 or anti-CD22 antibodies is generally well tolerated [11, 25], probably because these antibodies do not bind to bone marrow stem cells and other cells of the immune system, notably T cells. In contrast, tolerance of treatment with Campath antibody (alemtuzumab) is more compromised, since its target antigen is expressed on both normal B and T lymphocytes [26]. Campath treatment can therefore provoke depression of both antibody and cellular immunity, thereby frequently leading to severe infections.

An advantage of current RIT may be the fact that it is administered in combination with high amounts of non-labelled antibodies. The non-conjugated antibodies can themselves provide an efficient biological treatment of cancer or lymphoma and leukaemia [26–28]. Indeed, it is well documented that unlabelled monoclonal human IgG<sub>1</sub> antibodies provide highly efficient effector functions such as complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity or direct induction of apoptosis and cell killing. Current RIT is therefore a combination treatment that favourably combines biological therapy with radiation treatment.

Treatment repetition and/or prolonged application of internal emitters, as are used in conventional radiotherapy, might be a second requirement for efficient systemic radiation therapy. However, some currently used clinical antibodies are still of rodent origin (tositumomab, ibritumomab) [23, 24], bearing the risk of immunising patients and not allowing repeated treatment cycles. It has been shown, however, that chimerisation (rituximab) [27] and, even more so, humanisation of monoclonal antibodies (alemtuzumab or trastuzumab) [26, 28] significantly reduces the risk of a human anti-mouse IgG immune response. In peptide treatment and Auger electron therapy approaches, immune response to the targeting vehicles will generally not occur, providing the opportunity to repeat treatment cycles without particular constraints.

Targeting of a high percentage of all live cancerous cells may be a third requirement of systemic radiation therapy. It is important to realise that the tumour uptake of radiolabelled antibodies, peptides and other conjugates is frequently quite inhomogeneous. In this situation, the crossfire effect of  $\beta^-$  particle emitters can constitute a major advantage [9–12]. The longer range tissue penetration of this radiation type is due to a small energy deposition on the radiation path of only 0.2 keV/ $\mu\text{m}$ .  $\beta^-$  radiation is thus providing a low LET type radiation similar to X-rays. Owing to this long-range tissue penetration of  $\beta^-$  radiation, a crossfire effect is created, reaching a major portion of tumour cells rather homogeneously even in a solid tumour and despite inhomogeneous radiopharmaceutical distribution (Fig. 1). Another advantage of  $\beta^-$  radiation therapy approaches is the availability of multiple radioisotopes emitting low-, medium- and high-energy electron radiation (Table 1). These different energies provide short- to long-range tissue penetration paths of 0.1 up to 10 mm [29]. Optimal tumour sizes for given  $\beta^-$  energies have been modelled [30]. Results of *in vivo* studies, however, have not always correlated with these predictions [31, 32]. The disagreement may be partially due to insufficient knowledge of microscopic distribution of the radio-vector or insufficient coverage of radiobiological issues such as killing of clonogenic cells [31].

However, it is obvious that small tumour nodules or clusters of tumour cells will not be treated efficiently with high-energy  $\beta^-$  emitters. At a small target size, energy deposition of high-energy  $\beta^-$  radiation becomes predominant in the normal surrounding tissue and insufficient in the target tumour cells. As an example, it has been calculated



**Fig. 1.** Schematic presentation of  $\alpha$ ,  $\beta^-$  and Auger radiation path lengths in a cellular and subcellular environment (arbitrary scaling). Note that the major energy deposition of an Auger radiation decay occurs in the close vicinity of a few nm, while that of  $\alpha$  and  $\beta^-$  radiation occurs on tracks of 40–80  $\mu\text{m}$  and 0.1–10 mm, respectively

that the absorbed fraction per cell of electrons from  $^{90}\text{Y}$  with a mean energy of 935 keV would be as low as 0.1% [33]. In this respect, low-energy beta radiation would be more advantageous. Experimentally, superior anti-tumour efficacy was observed with somatostatin analogues labelled with a combination of a high- ( $^{90}\text{Y}$ ) and low-energy ( $^{177}\text{Lu}$ )  $\beta^-$  emitters in rats bearing simultaneously small and large tumours [34]. More efficient systemic radiation therapies against individual tumour cells or small cell clusters, with respect to radiation deposition in the target, would be provided with  $\alpha$  or Auger radiation emitters.

### Physical properties of Auger radiation

Auger radiation was described in 1925 by Pierre Auger [35]. Auger electrons arise from electronic shells of radionuclides when these decay by electron capture. Electron capture processes create inner shell electron vacancies by an electron transfer from this shell into the nucleus. The inner shell electron vacancies are subsequently filled by electron transitions from shells of higher

energy, a process that occurs in cascade. The energy difference of these transitions can be released either as photons or as low-energy electrons, the Auger electrons.

Typically, Auger radiation decays produce between 5 and 30 Auger electrons. Depending on the relationship of the electronic shells involved, these transitions are classified into Auger, Coster-Kronig and super-Coster-Kronig processes. The electrons emitted by these three processes are referred to as Auger electrons [19, 36–38].

Auger electrons are of low energy. The small negatively charged low-energy electrons produce multiple ionisations of high LET type (4–26 keV/ $\mu\text{m}$ ) and consequently have a very short range in biological tissues, typically <0.5  $\mu\text{m}$ . The large majority of Auger electrons have a tissue penetration range of the order of a few nm only [19].

Internal conversion (IC) electrons are created by collision of photons (from the nuclear decay) with inner shell electrons that are ejected. IC electrons have discrete energies in the keV range. This energy is higher than that of most Auger electrons as defined above but lower than that of classical  $\beta^-$  radiation such as emitted by  $^{131}\text{I}$  or  $^{90}\text{Y}$ . Some groups use a wider definition for Auger electrons, such that they comprise all low-energy electrons, and thereby consider that IC electrons are also among the Auger electrons. However, in contrast to Auger electrons, IC electrons produce mostly a low LET type tissue irradiation with a penetration path generally in the  $\mu\text{m}$  range. IC electrons from decays in the cytoplasm can accordingly reach the nuclear DNA. However, the observed biological efficacy of IC electrons is similar to that of conventional  $\beta^-$  radiation and X-rays [39]. When referring to IC electrons in this review, we shall call them by that name and make the distinction from Auger electrons, as justified by the different biological efficacy of these two radiation types.

A large number of radioisotopes emit some Auger radiation, but the most interesting Auger electron emitters for the purpose of treatment are  $^{125}\text{I}$ ,  $^{123}\text{I}$  and  $^{201}\text{Tl}$  (Table 2) [38].  $^{55}\text{Fe}$  would emit a very high proportion of radiation energy in the form of Auger electrons, but it does not qualify for therapy in humans owing to its half-life of 2.7 years. For the three mentioned emitters, the Auger radiation energy released per decay remains significant,

**Table 1.** Radiation properties of representative therapeutic radioisotopes

Radiation	$\beta^-$			$\alpha$	Auger electrons
	Low	Moderate	High	Very high	Very low
Examples	$^{169}\text{Er}$ $^{177}\text{Lu}$ $^{67}\text{Cu}$ $^{199}\text{Au}$ $^{33}\text{P}$	$^{131}\text{I}$ $^{186}\text{Re}$ $^{153}\text{Sm}$ $^{111}\text{Ag}$ $^{64}\text{Cu}$	$^{90}\text{Y}$ $^{188}\text{Re}$ $^{32}\text{P}$ $^{89}\text{Sr}$	$^{211}\text{At}$ $^{213}\text{Bi}$ $^{212}\text{Bi}$ $^{149}\text{Tb}$	$^{125}\text{I}$ $^{123}\text{I}$ $^{111}\text{In}$ $^{55}\text{Fe}$ $^{67}\text{Ga}$
Mean tissue range (mm)	0.1–0.3	0.3–1	1–5	0.03–0.08	<0.001
Particularity	Low LET and crossfire effect			High LET	High LET
Best suited for treatment of:	Tumour masses			Clusters and individual cells	Individual cells and clusters

**Table 2.** Examples of Auger electron emitters for which the Auger radiation represents a significant percentage of the overall energy release per decay

Isotope	Auger electrons/ decay	IC electrons/ decay	Auger energy/decay (keV)	IC energy/decay (keV)	Total energy/ decay (keV)	Auger energy in % of total energy/decay	IC energy in % of total energy/decay	$T_{1/2}$
$^{55}\text{Fe}$	5.1	0	4.2	0	5.8	71.9	0	2.7 yr
$^{67}\text{Ga}$	4.7	0.3	6.3	28.1	201.6	3.1	13.9	78 h
$^{99\text{m}}\text{Tc}$	4.0	1.1	0.9	15.4	142.6	0.6	10.8	6 h
$^{111}\text{In}$	14.7	0.2	6.8	25.9	419.2	1.6	6.2	67 h
$^{123}\text{I}$	14.9	0.2	7.4	20.2	200.4	3.7	10.1	13 h
$^{125}\text{I}$	24.9	0.9	12.2	7.2	61.4	19.9	11.8	59.4 d
$^{201}\text{Tl}$	36.9	1.1	15.3	30.2	138.5	11.0	21.8	73 h

Decay energies are given as reported by the AAPM nuclear medicine task group 6 [38]. X- and  $\gamma$ -ray contributions are only included in the column that shows the total energy release per decay

while it is frequently less than 1% for other isotopes like  $^{99\text{m}}\text{Tc}$ . For  $^{125}\text{I}$ ,  $^{123}\text{I}$  and  $^{201}\text{Tl}$ , the Auger electron energy release per decay represents between 3.7% and 19.9% of the total energy, a higher proportion of radiation energy being released in the form of photons. Since photons penetrate tissue with a low energy deposition per  $\mu\text{m}$  path, they are of minor relevance with respect to therapy but should continue to be borne in mind for the potential non-specific irradiation of radiosensitive tissues such as bone marrow.

The radioisotope  $^{123}\text{I}$ , though it releases only about 14% of its decay energy in the form of Auger and IC electrons, would be a good candidate for therapy. Its half-life of 13.2 h would be well adapted for peptide or oligonucleotide Auger radiation treatment and the biodistribution could be followed by scintigraphy based on the  $\gamma$  radiation of 159 keV.

### Energy deposition in the nuclear space

The shower of electrons produced in a typical Auger radiation decay has a punctiform origin. The abundance of low-energy electrons in a microscopic or “nanoscopic” space can be seen as an onion-like energy deposition in concentric spherical shells. It has been calculated that the absorbed energy around the decay site of  $^{125}\text{I}$  would be  $80 \text{ eV/nm}^3$  within the first nanometre. Energy deposition would drop to about  $10 \text{ eV/nm}^3$  in the second nanometre and fall further to  $3 \text{ eV/nm}^3$  in the third nanometre [40]. The calculated energy deposition of  $10 \text{ eV/nm}^3$  within a radius of 2 nm implies a locally absorbed radiation dose of 1.6 MGy [40]. This local radiation dose is 22,000 times higher than a conventional external beam radiation dose of 70 Gy delivered in conventional radiation therapy to tumours over several weeks. The locally deposited energy of 1.6 MGy corresponds to  $1.6 \times 10^{-18} \text{ J/nm}^3$ . This translates into a temperature increase of  $382^\circ\text{C}$  per  $\text{nm}^3$  of water. Obviously, this locally very high energy deposition within 2 nm from decay will then rapidly dissipate, i.e. dilute, into the total space of the nucleus. If we consider a nucleus of 2  $\mu\text{m}$

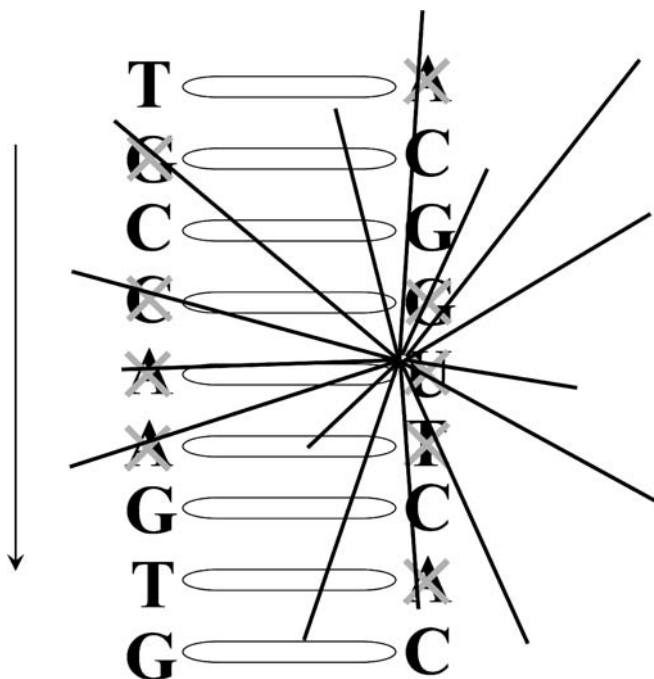
radius, the dilution factor would be  $10^9$  and the overall heating effect for the nucleus would be  $0.38 \times 10^{-6}^\circ\text{C}$  and thus insignificant.

### Auger radiation biology

The double strand DNA helix presents a diameter of 2 nm. In a typical Auger radiation decay, the highest energy deposition occurs in spheres of 1–2 nm, as described elsewhere [40]. This means that the calculated local energy deposition of an Auger emitter incorporated into DNA would hit both DNA strands with an energy of 1.6 MGy or higher. This radiation energy is therefore largely sufficient to disrupt both DNA strands over distances of several nucleotides (Fig. 2) [39, 41]. For  $^{125}\text{I}$  or  $^{123}\text{I}$  decays associated with DNA, this translates into a general rule of: “One decay=one double strand break”. Similar to an  $\alpha$  radiation path through the cell nucleus, the genetic information is lost in these double strand breaks owing to destruction of several nucleotides on both strands [42–44]. Repair still remains possible but will frequently be erroneous. These features are responsible for the high RBE of Auger radiation when decays occur in close association with DNA. It is a most intriguing feature of Auger radiation that it possesses the high cytotoxicity of  $\alpha$  radiation when occurring in close vicinity to DNA while being of low toxicity outside the cell nucleus.

Besides the direct effect of Auger electrons on DNA double strands, an indirect radiation effect of Auger energy deposition will occur via production of radicals [45]. The radicals diffuse freely in the intracellular space and can cause further DNA damage. Even a bystander effect by diffusion of radicals through gap junctions has been described [46].

In order to compare the RBE of different radiation types, the radiation weighting factor ( $W_R$ ) has been introduced.  $W_R$  compares the biological efficacy of a given radiation dose of a particular radiation type with that of X-rays. X-ray effects are being attributed the  $W_R$  of 1.  $^{125}\text{I}$ - and  $^{123}\text{I}$ -iododeoxyuridine (IdUrd) has frequently been used to



**Fig. 2.** Schematic presentation of an Auger radiation decay in a stretch of double strand DNA.  $^{125}\text{I}$ -iododeoxyuridine ( $^*\text{U}$ ) is incorporated in DNA as a thymidine (T) analogue. The *arrowhead* indicates the reading direction of a given gene. The destruction of the antisense sequence guanine-iododeoxyuridine-thymidine (G- $^*\text{U}$ -T) goes together with the destruction of the sense sequence cytidine-adenine-adenine (C-A-A), whereby the genetic code is lost over a stretch of three nucleotides

measure the biological effects of Auger radiation. Since the decay sites of radio-IdUrd, once incorporated into DNA, fulfil the requirement of close vicinity to DNA, double strand breaks occur with a probability of about 1 per decay. The RBE of  $^{125}\text{I}$ -IdUrd and  $^{123}\text{I}$ -IdUrd measured in V79 cells was 8 and 7 [40, 47], respectively. This means that, for an identical deposition of energy, the Auger radiation of DNA-incorporated  $^{125}\text{I}$ -IdUrd and  $^{123}\text{I}$ -IdUrd would be eight- and sevenfold more efficient compared with X-rays,  $\gamma$  or conventional  $\beta^-$  radiation [48]. This biological efficacy of DNA-incorporated  $^{123}\text{I}/^{125}\text{I}$ -IdUrd would be similar to that of  $\alpha$  radiation [48, 49]. Nuclear localised Auger processes that are not directly linked to DNA would, however, develop a  $W_R$  only about half that of DNA-associated decays [39].

In experiments other than those mentioned above, different research groups have reported widely divergent biological efficacies of Auger radiation, with  $W_R$  values as high as 64 in the particular situation of chronic irradiations [50]. The appreciation of these and other reported effects remains a matter of debate [49]. The Nuclear Medicine task group of the American Association of Medical Physicists has proposed the application of a radiation weighting factor of 10 for deterministic effects of Auger radiation and a factor of 20 for stochastic effects, provided that this radiation occurs in the nucleus in close association with DNA [39]. In view of the mentioned divergent observations, this proposal is not generally accepted.

Unlike most Auger electrons, IC electrons may significantly irradiate the cell nucleus when decaying in the cytoplasm. Because of the lower biological efficacy of IC electrons, however, the predicted radiation effects must be clearly distinguished between IC and Auger electrons, notably when the latter occur in the nucleus. As previously mentioned, such IC electrons develop a low LET type irradiation associated with mostly single strand breaks and a  $W_R$  of about 1 [39].

### Gonadal effects

It is quite well established that Auger irradiation of the gonads also gives an RBE of about 7 regarding deterministic effects [51]. However, the stochastic effects of Auger radiation emitters in human gonads remain a question of debate. There are divergent observations concerning not only long term effects but also the calculation of dose deposition in gonad cells. It has been reported that the irradiation dose to sperm heads may be up to 160 times higher than that cited by ICRP report 53, which did not include Auger radiation [52]. Furthermore, transfer of  $^{111}\text{In}$ -labelled transferrin through the intact, so-called blood-testis barrier has been reported, which would be mediated by the Sertoli cells [53, 54]. The authors referred to evidence from experimental animal studies that the emitted Auger radiation might even produce hereditary effects in offspring [55]. It could be hypothesised that similar situations would be found in ovaries, since separation of the maternal and offspring circulation would provide a barrier similar to that in male gonads. As a consequence of these observations, these authors [55] suggested that the administration of larger diagnostic activities of  $^{111}\text{In}$ -radiopharmaceuticals might require contraception. In comparison, our first experiments with  $^{125}\text{I}$ -IdUrd did not indicate any significant uptake into gonads of mice. This suggested that the blood-testis barrier might be operational for this agent as well. This statement would be in agreement with the observation that radio-IdUrd does not cross the intact blood-brain barrier [56]. However, autoradiographic experiments have not been performed in these studies, which represents a limitation.

### Bystander effect

It has been reported that an *in vivo* bystander effect occurred in experiments using mixtures of  $^{125}\text{I}$ -IdUrd-labelled LS174T cells and untreated cells [57]. The existence of such an effect would allow therapy to be envisaged even if not all cancer cells were targeted with a given radiopharmaceutical. In the mentioned experiments, the mixed cell suspensions containing defined proportions of  $^{125}\text{I}$ -IdUrd pre-labelled cells and a constant amount of unlabelled cells were injected subcutaneously in nude mice and tumour outgrowth was followed 10–15 days later. It appeared that the radiolabelled, dying cells developed a growth inhibitory effect on the non-pre-labelled cells, producing a tumour growth delay 2–4 days longer than expected. It was also

shown that the growth delay could not be explained by a minor radiation exposure of non-pre-labelled cells from the  $\gamma$ -radiation of  $^{125}\text{I}$ , since much higher external beam irradiation doses were required to diminish tumour growth to a similar degree. The authors thus concluded that an Auger radiation therapy effect was observed which involved more than just the tumour cells that were pre-labelled. Whether this observation in fresh transplanted cells can be extrapolated directly to outgrown tumours targeted with Auger radiation emitters remains to be shown.

## Dosimetry

Biodistribution and kinetic measurements of internal radiopharmaceuticals in patients are difficult to achieve. A high-quality dosimetry of  $\gamma$ - or positron-emitting radiopharmaceuticals remains most difficult to perform in a standard clinical setting [58]. The task becomes close to unrealistic on an individual patient basis for Auger radiation emitters. For Auger radiation dosimetry, knowledge of the percentage of cells that are targeted in individual tissues is essential. Furthermore, most relevant Auger irradiation would occur only for DNA-associated emitters [39, 59]. In contrast to target cells, non-target cells would not be, or would be only minimally, irradiated from Auger electron decays.

Given that the cumulated organ activity is known, a mean nuclear absorbed radiation dose may be calculated, if the percentage of DNA-associated Auger radiation can be estimated. S values for such situations have been provided [60]. For the estimation of the risk of long-term stochastic effects, a  $W_R$  of 20 may then be applied for nuclear-associated Auger radiation [39]. This may provide a radiation dose risk estimate for a patient exposed to Auger radiation in a nuclear medicine diagnostic procedure [61]. As a best approximation, this dosimetry was based on biodistribution and DNA incorporation measurements performed in mice [61]. Measurement of all these parameters in individual patients would be impossible. Such risk appreciation should, however, be taken seriously, particularly if significant uptake of the radiopharmaceutical occurs in the gonads. As mentioned, if an Auger radiopharmaceutical is taken up by the gonads, the cell-specific localisation would have to clearly distinguish between germline and stromal cell localisation in order to allow for adequate Auger radiation dosimetry.

For calculation of deterministic, cytotoxic effects of Auger radiation, knowledge of a mean nuclear absorbed radiation dose in an organ, however, is insufficient. Here, it is essential to have an estimate of the percentage of cells that are targeted and to know the uptake rate of the radiopharmaceutical into these cells. Furthermore, considering the effects of Auger radiation emitters with a long half-life, such as  $^{125}\text{I}$ , cells insufficiently targeted might escape cytotoxicity through cell division [36]. Cell division produces a dilution of Auger radiation decays into multiple daughter cells. Furthermore, considering clonal survival, the probability of survival of at least one out of several

daughter cells will be higher than that of a single parent cell exposed to the same number of Auger decays. The precise dosimetric handling of cell division during long-lasting Auger radiation is therefore highly complex. It requires, in addition to the mentioned considerations, attention to the notion of cell division delay [62] due to continued irradiation that causes double strand DNA breaks. Double strand breaks in turn induce cycling stop signals at different check-points of the cell cycle allowing repair.

Biological Auger radiation effects have frequently been measured with  $^{125}\text{I}$  or  $^{123}\text{I}$ -IdUrd. The short, 13-h half-life of  $^{123}\text{I}$  provides an Auger radiation emitter that delivers the large majority of its irradiation within 1 day, while cellular doubling of tumours frequently occurs within 1 to several days. In contrast, due to the long, 60-day half-life,  $^{125}\text{I}$ , radiation delivery will occur over several days or weeks provided that the radiopharmaceutical remains associated with DNA. It was calculated for  $^{125}\text{I}$ -IdUrd that a given cell was sterilised with a mean of 120 Auger radiation decays that occurred in close association with DNA [62]. This would correspond to about 120 DNA double strand breaks. This calculation was based on the knowledge of a normal cell division time as well as a division time under a given Auger irradiation condition. Thus, the out-dilution effect of  $^{125}\text{I}$ -IdUrd during cell division was taken into account in this dosimetry. However, in our understanding, the creation of two or multiple daughter cells with an accordingly higher clonal survival probability compared with a single cell has not been included in the mentioned calculations [36, 62]. Overall, the above-mentioned number of 120 Auger decays that would kill a cell therefore remains an estimate and applicable to the particular cell line only.

## Radioprotection

In view of the difficulty of precise Auger radiation dosimetry, radioprotection in this field requires the application of a large factor of potential overprotection in order to cover the uncertainties from the dose calculation. For any given Auger electron-emitting radiopharmaceutical, a potential localisation in the cell nucleus of bone marrow stem cells or, notably, germinal cells should be of the utmost concern. This could be the case for oligonucleotides,  $^{125}\text{I}$ -IdUrd or other nucleotides as well as hormones. However, nuclear localisation might also occur with other radiopharmaceuticals, as has been described for  $^{111}\text{In}$ -labelled pharmaceuticals [53, 54]. According to these authors,  $^{111}\text{In}$  might be released from a radiopharmaceutical and be transported into gonad cells via binding to transferrin.

## Experimental treatment approaches

### *Hormones and growth factors*

Steroid hormones and analogues localise to nuclear receptors [63, 64]. An advantage of hormonal vectors is

the fact that all tumour cells are potential targets.  $^{123}\text{I}$ - and  $^{125}\text{I}$ -iodo-oestrogens were used to study Auger radiation treatment of oestrogen receptor-positive MCF-7 breast cancer cells [65–67]. The authors determined the dose producing on average one lethal event per cell, the  $D_{37}$ . The random distribution of one lethal event per cell predicts that 37% of cells will survive, as calculated by a Gaussian model [17]. This leads to the definition of the term  $D_{37}$  as the percent of survival corresponding to a mean of one lethal hit per cell. These authors determined a  $D_{37}$  of 28 decays/cell for  $^{125}\text{I}$ -oestrogen. Similarly, for  $^{125}\text{I}$ -IdUrd the  $D_{37}$  was determined as 30 decays/cell. In these experiments, cells were frozen in order to accumulate the number of decays/cell, facilitating dosimetry calculations without interference from cell division. In terms of biological efficacy, these numbers of decays would correspond to an RBE of about 4.5. However, freezing as an artificial event might itself modulate the survival probability after a given cell insult and the biological relevance of this determination of  $D_{37}$  might therefore not be definitive.

Epidermal growth factor (EGF) has been labelled with  $^{111}\text{In}$ -DTPA. It was reported that after an incubation period of 24 h up to 9.6% of the conjugate was found associated with nuclear chromatin of breast cancer cells overexpressing the EGF receptor [68]. Growth inhibition was observed in a corresponding tumour xenograft model [69].

With regard to  $^{111}\text{In}$ -DTPA-labelled octreotide, autoradiographic *in vitro* experiments showed that a small fraction was being incorporated into the cell nucleus of receptor-positive cells [70]. With the aim of increasing the affinity to receptors, experimental studies with different somatostatin analogues have been performed, including recently a tri-functional peptide [71]. Based on the observation of partial intranuclear localisation and evidence of therapeutic efficacy in rats,  $^{111}\text{In}$ -DTPA-octreotide has been evaluated in clinical studies for therapy of neuroendocrine tumours [72–75], as discussed below.

### *Radio-iododeoxyuridine (IdUrd)*

Radiolabelled iododeoxyuridine, such as  $^{125}\text{I}$ - and  $^{123}\text{I}$ -IdUrd, has been studied for many years for its Auger radiation effects [19]. The fact that these thymidine analogues are directly incorporated into nascent DNA during the synthesis phase of the cell cycle provides a most reliable model for the experimental measurement of Auger radiation biological effects.

A first major restriction of radiolabelled nucleotides, however, lies in the fact that they are incorporated into DNA only during the synthesis phase of the cell cycle. This restriction has generally been overcome *in vitro* by using incubation times covering at least two to three cell cycles. For *in vivo* applications, osmotic pumps delivering radio-IdUrd continuously over several days have been used in order to bypass the problem of the very short circulation time of nucleotides that are rapidly degraded after administration.

A low rate of DNA incorporation both *in vitro* and *in vivo* constitutes a second significant problem with the use of  $^{125}\text{I}$ - or  $^{123}\text{I}$ -IdUrd. There are two obvious major reasons for this: first, rapid catabolism of IdUrd and second, competition from endogenous thymidine (dThd). In order to circumvent the low DNA incorporation rate of  $^{125}\text{I}$ - and  $^{123}\text{I}$ -IdUrd, incorporation modulation using dThd synthesis inhibition has been studied by several groups [76–78]. The aim has been to take advantage, under blocked endogenous dThd synthesis, of the preferential incorporation of radio-IdUrd into DNA through the salvage pathway. The combination with different dThd synthesis inhibitors, such as fluorodeoxyuridine (FdUrd) or methotrexate, produced up to a fivefold increase in the incorporation rates of unlabelled or radiolabelled IdUrd or bromodeoxyuridine [76–78]. However, the increase in incorporation after dThd synthesis inhibition did not yield the expected increase in therapeutic efficacy of radio-IdUrd [77]. The reason for the absence of an increase in toxicity corresponding to the increased DNA incorporation rate of  $^{125}\text{I}$ -IdUrd after dThd synthesis inhibition remains unexplained, to our knowledge.

We used the approach of short exposure of radio-IdUrd in combination with short, non-toxic exposure to FdUrd. We first showed that  $^{125}\text{I}$ -IdUrd incorporation in DNA increased with this approach multiple-fold both *in vitro* and *in vivo* using three different glioblastoma cell lines and tumours [79]. After direct intra-tumoural application, up to 20% of radio-IdUrd was incorporated into tumour cell DNA after FdUrd pre-treatment [80]. In comparison, only 4% of injected activity was DNA incorporated in non-pre-treated animals. In the next step, we improved targeting of radio-IdUrd into a higher number of cells. After the non-toxic pre-treatment with FdUrd, cell synchronisation into early S phase was observed for the different cell lines studied 16–24 h post FdUrd. Thus  $\geq 70\%$  of glioblastoma cells were synchronised in S phase and incorporated high amounts of radio-IdUrd [81] at these delayed times. In our most recent experiments, we were able to show that 70–80% of glioblastoma cells were killed after a single incubation with modest activities of  $^{125}\text{I}$ -IdUrd after FdUrd pre-treatment, whereas maximally 30% of cells were killed after exposure to  $^{125}\text{I}$ -IdUrd alone, even when using much higher concentrations. These percentages thus correlate with the percentage of S phase cells targeted after FdUrd pre-treatment.

*In vivo*, prolonged TS inhibition has been observed after treatment with 5-fluorouracil [82]. Furthermore, *i.v.* push injection of FdUrd is overall of low toxicity and has been used therapeutically in the early stage of clinical application of FdUrd [83, 84]. In comparison, the maximal tolerated dose (MTD) of continued exposure (0.15 mg/kg FdUrd perfused per day) is about 200-fold lower than the MTD of FdUrd given in a single *i.v.* push injection (30 mg/kg per day). These data suggest that the approach of FdUrd-induced cell synchronisation might be used *in vivo* to deliver radio-IdUrd to a high percentage of tumour cells.

As another parameter of radio-IdUrd biology, the function of nucleoside transporters has occasionally been

studied [85]. Certain subtypes of nucleoside transporters are known to be expressed in a cell cycle-dependent manner. Thus, hCNT1 is increased in G<sub>1</sub>-S transition [86]. It has been shown that inhibition of thymidylate synthase leads to multiple-fold (30-fold) up-regulation of nucleoside transporter expression [85]. This observation appears compatible with the description of increased hCNT1 expression in the synthesis phase of the cell cycle [86]. In our opinion, such a regulation feedback would favour cell uptake and incorporation of exogenous nucleosides under the condition of suppressed endogenous dThd synthesis and might represent a key element in certain Auger radiation therapy approaches, such as intra-tumoural injection of radio-IdUrd.

### *Oligonucleotides*

Targeting of specific sequences of DNA or mRNA with triplex-forming oligonucleotides [44, 87, 88] or antisense oligonucleotides [89, 90] is a further appealing perspective of Auger radiation treatment. The strategy aims to target Auger radiation into key elements of cell survival. Targeting of genes that are overexpressed in tumours would be most interesting. As with nucleotides, however, this strategy faces the obstacles of oligonucleotide instability and low rates of uptake into the nuclear target. The transfer of the oligonucleotides from the cytoplasm into the nuclear compartment is generally very limited and appears to be controlled by cellular elements that have not been identified [44].

Aptamers represent another class of DNA or RNA oligonucleotides. Aptamers fold into unique tertiary conformations that are capable of binding most diverse target antigens with high affinity and specificity, analogous to antibodies [91–93]. Tumour targeting with a radiolabelled aptamer has been demonstrated [94]. This opens the possibility of using them in an internal radiotherapy strategy. However, in common with the other oligonucleotides, the *in vivo* half-lives of aptamers are generally short. Different stabilisation strategies have therefore been attempted to overcome this limitation [92, 95–97].

Oligonucleotides can target mRNA in cytoplasm and in the nucleus. Auger radiation delivered with antisense oligonucleotides might be a means to disrupt mRNA and thus be aimed at reducing translation of particular genes. This approach could therefore be a means of circumventing the problem of low uptake into the nuclear space. Similarly, aptamers can directly target gene products in cytoplasm and coupling with Auger emitters could potentially be a means to increase their efficacy.

### *Low-energy electrons targeted into cytoplasm*

Different monoclonal antibodies elicit antigen internalisation upon binding. Such antibodies have been labelled with <sup>125</sup>I and other Auger or low-energy electron emitters [98–102]. Two-step targeting has been used in this approach

as well [103]. Similarly, MIBG, as an analogue of nor-adrenaline, is concentrated in adrenergic tumour cells and can be labelled with the different iodine radio-isotopes for treatment purposes [104]. <sup>125</sup>I-labelled MIBG has been used in an Auger/low-energy electron therapy approach [105]. The fraction of intracellular generated electrons from <sup>125</sup>I decays (18-keV electrons) that is absorbed by individual cells has been calculated to be as high as 60%. In comparison, the absorbed fraction per cell of electrons from <sup>90</sup>Y, with a mean energy of 935 keV, would be as low as 0.1% [33]. Other low-energy electron emitters have also been proposed for targeting of small tumour cell clusters, such as <sup>58</sup>Co and <sup>103m</sup>Rh [106] or <sup>67</sup>Ga- and <sup>111</sup>In-labelled compounds [102].

<sup>125</sup>I-iodide has been explored for therapy of hyperthyroidism and thyroid cancer [107–111]. However, no significant advantage could be observed as compared with <sup>131</sup>I therapy. Radio-targeted gene therapy is a similar approach whereby new genes are introduced into a tumour, allowing cellular uptake of Auger radiopharmaceuticals. Tumour transfection with the sodium iodide symporter followed by treatment with <sup>125</sup>I could constitute such a low-energy electron radiation therapy approach [112, 113]. The biological efficacy to be expected from such approaches will be similar to that of X-rays for an identical radiation dose deposition.

### **Clinical studies**

Treatment studies of thyroid cancer and hyperthyroidism have been performed with <sup>125</sup>I [108–111]. The combination of longer range, low-energy,  $\gamma$  radiation with the short-range Auger and IC electron effects of <sup>125</sup>I could be a means to treat both large and small cancer nodules or hyperplastic thyroid tissue. The efficacy of <sup>125</sup>I treatment, however, has been limited. The fact that radio-iodine does not enter the nucleus and stays only briefly in the cytoplasm before being deposited in colloidal form might be responsible for the low contribution of Auger effects in treatment. In fact, only a minor portion of the low-energy electrons from <sup>125</sup>I decay in cytoplasm and particularly in colloid can reach the nuclear DNA. Furthermore, the radiation protection issues of <sup>125</sup>I are difficult to handle in the clinical setting because of its long half-life of 60 days and continued urinary elimination.

Most advanced therapy studies have been performed with <sup>111</sup>In-DTPA labelled octreotide [72, 73]. Here, nuclear localisation of <sup>111</sup>In-octreotide had been described, though the percentage entering the nucleus might be small overall [70]. A direct Auger radiation effect on DNA was therefore expected. Studies were performed on more than 50 patients, but efficacy was rather modest. Therapeutic effects after injection of 20–160 GBq <sup>111</sup>In-DTPA-octreotide consisted mostly in stabilisation of tumour growth, partial remissions remaining scarce. Bone marrow toxicity was generally mild; however, two myelodysplastic syndromes and one case of leukaemia were observed in a group of six patients who had been treated with a total of more than 100 GBq <sup>111</sup>In-DTPA-



octreotide [74, 75]. This latter observation was rather unexpected. A word of caution was expressed by the authors, who suggested that cumulated activities of  $^{111}\text{In}$ -peptide conjugates should not exceed 100 GBq. A dosimetric calculation for these clinical therapy studies has not been presented, to our knowledge. Insufficient knowledge of biodistribution and subcellular localisation of the Auger radiation emitter might have been responsible for this omission. The reason for the limited efficacy of these treatments therefore remains a matter of speculation. In fact, it was observed that the distribution of radiotracer was quite inhomogeneous in kidney [114]. Similar inhomogeneous distribution of radiotracer might have occurred in tumour and have been partially responsible for the limited therapeutic efficacy. In more recent studies, these groups based their systemic radiation therapy of neuroendocrine tumours on  $\beta^-$  emitters using  $^{90}\text{Y}$ - or  $^{177}\text{Lu}$ -labelled somatostatin analogues [75].

Clinical studies with radio-I<sup>125</sup>Urd have been performed, mostly with the aim of measuring and visualising tumour targeting [56, 115–118]. Multiple injections or prolonged perfusion was not used in these rather preparative clinical approaches. The requirement that tumour therapy with Auger electron emitters should target a high percentage of all cancerous cells was therefore not met. As a consequence, therapeutic efficacy was generally not observed in these studies.

Antibodies directed against different tumour-associated antigens might elicit antigen internalisation upon binding. Such antibodies have been labelled with  $^{125}\text{I}$  and other Auger or low-energy electron emitters, and first therapy studies been performed in patients [98, 119]. Similarly,  $^{125}\text{I}$ -labelled MIBG has been used in an Auger/low-energy electron therapy approach [105]. Therapeutic efficacy with these latter agents was mostly modest. In fact, also in radio-MIBG treatment,  $^{131}\text{I}$  is still the preferred radioisotope for therapeutic applications [104]. As for radioimmunotherapy of solid tumours based on  $\beta^-$  emitters, bone marrow toxicity has been revealed as the dose-limiting factor for antibodies labelled with Auger radiation emitters. Furthermore, and similar to thyroid treatment with  $^{125}\text{I}$ , the internalising antibodies do not enter the nucleus. The Auger radiation therefore reaches the nucleus only partially, and its biological efficacy will be modest, with a  $W_R$  of about 1 [39].

Overall, clinical studies with Auger radiation emitters have not met the major goal of efficacy, but some of these studies have been performed with the aim of gaining knowledge as to the feasibility of such an approach, rather than with a therapeutic objective. Once the problems have been solved, however, the particular properties of Auger radiation will make its use for therapy an attractive proposition.

### Conclusion

Targeting of Auger or IC electron emitters into the nucleus or cytoplasm of tumour cells is an appealing approach for

systemic radiation therapy. Multiple obstacles to the administration of such therapies have been recognised and some of them have been partially overcome. However, most of these approaches still await demonstration of successful use in animal models, which will be essential prior to clinical application. Better understanding of Auger radiation dosimetry and of its biological efficacy remains another major task. The latter must guide our therapy research, but in addition information must be gathered on many radiopharmaceuticals that can reach the cell nuclei with potential harmful Auger radiation. The combination of these efforts may allow the potential health risk of this particular radiation type to be assessed and may also guide research in an appropriate way towards tumour therapy.

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