The quest to exploit the Auger effect in cancer radiotherapy – a reflective review

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ABSTRACT
To identify the emergence of the recognition of the potential of the Auger effect for clinical application, and after tracing the salient milestones towards that goal, to evaluate the status quo and future prospects. It was not until 40 years after the discovery of Auger electrons, that the availability of radioactive DNA precursors enabled the biological power, and the clinical potential, of the Auger effect to be appreciated. Important milestones on the path to clinical translation have been identified and reached, but hurdles remain. Nevertheless the potential is still evident, and there is reasonable optimism that the goal of clinical translation is achievable.

Introduction
This review is not intended to be comprehensive or up-to-date; rather, it is the reflections of two overlapping personal journeys that have at times included forays into the subject of this review. We aim to recount what seemed to us to be the major stepping stones towards the goal of translating the Auger effect into a therapeutic modality. In the introductory lecture at the Kyoto Symposium, this goal was referred to as the ‘Treasure’. The allegory was further extended to representing some of the key steps towards the goal as maritime voyages to a tropical island (Auger Island) in search of the hidden treasure.

Unavoidably this is a biased view (albeit in two dimensions), and we apologise in advance for those important contributions that we have not included. Likewise our perception of the key conceptual steps is subject to debate. Where possible we have referred the reader to more comprehensive reviews of particular aspects. We also dwell on the personalities that have been involved in this quest using as a framework, the gathering of the Auger community every 4 years in satellite symposia of the International Congress of Radiation Research (Table 1), starting with the meeting in Oxford in 1987 (Figure 1). Also listed in Table 1 were two prior meetings which were important in germinating the Auger community. These early meetings were discussed briefly at the Jülich Symposium by one of us (Feinendegen 2012), but are described again here for the sake of cohesion.

The microdosimetry symposium in Jülich in 1975 is regarded as the foundation of the Auger symposium series, but even before that, there was a meeting in Vienna from 9–13 October 1967 on ‘Biological effects of transmutation and decay of incorporated radioisotopes’ (Figure 2). The introductory and broad review at this meeting by one of the current authors (LEF) on ‘Problems associated with the use of labelled molecules in biology and medicine’ (available online in the Supplementary Material) included a paragraph on the Auger effect (Feinendegen 1968). It states a lack of knowledge regarding the consequences of Auger effects in biological systems, especially the DNA in contrast to available knowledge on the Auger effect in radiation-chemistry. Here experiments had shown that, for instance, the induction of photon-induced cascades of electrons in iodine by soft X-irradiation of CH$_3$I led to molecular disruption (Carlson & White 1966). The nature of this disruption fitted the hypothesis that charge transfer processes in the molecular ion with subsequent charge transfer and distribution decomposes the molecule violently by Coulombic forces. Biological effects from the Auger emitter $^{125}$I thus need be considered as a result of both low energy electrons and localized molecular charge transfers. The Vienna reviewer, one of the authors (LEF), had worked for years with $^{125}$I-iododeoxyuridine ($^{125}$I-UdR) as tracer for DNA and DNA synthesis, using the Auger electrons for micro-autoradiography and the photon emission for external counting, for instance in whole body rodents. From reading the results of the radiation-chemical experiments, the need to study the radiobiological consequences and usefulness of the Auger effect was recognized, especially in DNA of mammalian cells, as represented in Figure 2. Experimental work began immediately thereafter in Jülich (Ertl & Feinendegen 1969), and elsewhere. Notice that this is more than 40 years after the description of the Auger effect.

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For those readers not familiar with the details of the Auger phenomenon, a nice outline can be found in the proceedings of the Boston Symposium (Howell 2008), and in more detail elsewhere (Charlton & Booz 1981; Pomplun et al. 1987). Auger’s experiments involved study of photos of photoelectron tracks visualized using a Wilson Cloud chamber. They show the very low energy, short tracks from the Auger cascade following photo-ionization. The experiments are described in the publication of a lecture he gave late in life (Auger 1975). Apart from reproductions of photos of tracks of photo- and Auger-electrons, his review also includes an old photo of the equipment that he used. A much clearer picture of a Wilson Cloud chamber apparatus, in the Museum at the Cavendish Laboratory in Cambridge, is accessible on the internet.

The work of Pierre Auger was not the whole story of the discovery of what we now know as the Auger effect. Lisa Meitner is credited with the independent discovery of the effect in 1922 (Meitner 1922), before Auger (Auger 1923). At that time she was already working in Berlin with physicist Otto Hahn, who in 1944 was awarded the Nobel Prize for Chemistry for discovery of nuclear fission in December, 1938. Meitner is often mentioned as one of the examples of women who were overlooked by the Nobel Committee (Bentzen 2000; Duparc 2009). We perhaps should acknowledge that Pierre Auger may have received more than his share of the credit for the discovery of what we now call Auger electrons, although this has been disputed (Duparc 2009).

### Early biological experiments

The crucial link of the Auger effect with biology undoubtedly came with the ability to incorporate radioactive elements into DNA. The first example was tritiated thymidine (usually incorporated into the methyl group), which features on the cover of the proceedings of the 1967 meeting in Vienna (Figure 2). DNA polymerase hardly notices the difference when the methyl group is replaced with an iodine atom having a similar radius, and thus allows the incorporation of $^{125}$I into DNA. Within 2 years after the Vienna meeting, the first experiments in the whole body of mice compared the toxicity of $^{125}$I- and tritium in DNA. The degree of $^{125}$I toxicity surpassed that of tritium by a several fold larger value than expected on the basis of cell-doses from tritium beta and Auger electrons (Ertl & Feinendegen 1969). Also early on, seminal experimental data in parallel on mouse bone-marrow cells in vivo, a number of mammalian cells in culture, and bacteriophages largely in Europe, in Israel and the USA unravelled the:

1. Extraordinary biological toxicity of the Auger effect compared to that from beta-particles and external low Linear Energy Transfer (LET) irradiation;
2. Cellular dosimetry and the particular consequences of the Auger effect in DNA of mammalian cells and microorganisms;
3. Limited repair of Auger effect-induced DNA damage;
4. Particular sensitivity of the late-S-phase DNA to damage by the Auger effect;
5. Relative toxicities of the Auger effects in different molecular positions and cell sites, and
6. Potential of the Auger effect for tumour therapy.

Amongst the earliest reports, summarized in Feinendegen (1975) showing the amazing cytotoxicity of iodine-125 decay in DNA, was one that compared iodine-125 with iodine-131 and tritium, on a per decay/cell basis (Hofer & Hughes 1971); reproduced in Figure 3. Incidentally, Kurt Hofer’s engaging personality, and boundless enthusiasm for the Auger effect was infectious, and he certainly inspired one (RFM) of the authors.

A few years later, $^{125}$I-UdR was incorporated into bacteriophage DNA growing in *Escherichia coli*, and estimation of the average molecular size of DNA by sedimentation in sucrose gradients revealed that on average, each decay event yields a double-stranded break (DSB) (Krisch & Sauri 1975). In these bacteriophages, the decay event also corresponds to a lethal event. Interestingly, this came after the first experiments in mammalian cells, showing that a lethal event corresponds to about 50 decays per cell nucleus (Burki et al. 1973).

$^{125}$I-iododeoxyuridine as a therapeutic agent . . . and then $^{125}$I-labelled Tamoxifen

The remarkable cytotoxicity of iodine-125 decay in DNA, combined the simple idea that $^{125}$I-iododeoxyuridine would be incorporated selectively into dividing cells, immediately suggested a potential therapeutic strategy, assuming some measure of selectivity for rapidly dividing tumours. The results of experiments with tumour-bearing mice, published in *Nature*, led Bill Bloomer and Jim Adelstein (Bloomer & Adelstein 1977) to the conclusion that the idea ‘provides the basis for a new approach to the treatment of cancer.’ Figure 4 shows Bill Bloomer and Jim Adelstein at the Jülich meeting in October 1975, about a year before they submitted their paper to
Figure 1. Participants at the 1st Auger symposium at Charney Manor, Oxfordshire, on 17 July 1987. Of the listed participants, A. Edwards (UK), C. McIntyre (UK) and H. Nikjoo (UK) are known to have been absent from the photo, and a further two of D. Ackery (UK), R. Carpenter (UK), R. Ellis (UK) and A. Wilson (UK), were apparently absent, and the other two unidentified in the photo.

1. D. Rao (USA) 2. S. Apelgot (France) 3. A. Yunis (UK)
4. J. Adelstein (USA) 5. K. Johanson (Sweden) 6. L. Yasui (USA)
7. D. Charlton (Canada) 8. V. Mylavarapu (USA) 9. R. Howell (USA)
10. R. Hamm (USA) 11. K. Sastry (USA) 12. A. Harrison (UK)
13. K. Hieda (Japan) 14. S. Sundall-Bergman (Sw.) 15. A. Halpern (Germany)
16. K. Baverstock (UK) 17. H. Maezawa (Japan) 18. Y. Fujiwara (Japan)
19. G. Ludwikow (Sweden) 20. A. Kassis (USA) 21. R. Carpenter (UK)
22. K. Kobayashi (Japan) 23. L. Denison (Australia) 24. T. Ito (Japan)
25. W. Rau (Germany) 26. C. Lucke-Huhle (Germ.) 27. I. Radford (Australia)
28. K. Bagshawe (UK) 29. R. Martin (Australia) 30. R. Fairchild (USA)
31. R. Cundall (UK) 32. – 33. I. Kaneko (Japan)
34. D. Goodhead (UK) 35. J. Humm (UK) 36. A. Al-Kazwini (UK)
37. – 38. H. Ohara (Japan)
Nature. Clearly this was a landmark study and certainly the first step towards the goal of translation of the Auger effect. However, a strategy based on the rate of cell division is unlikely to provide an exploitable therapeutic ratio except in specific instances, one of which is advanced malignant meningitis (Kassis et al. 2004).

The Boston group were quick to realize that more sophisticated biological targeting was required. They used \(^{125}\)I-labelled Tamoxifen, a drug that binds to estrogen receptors, which is over expressed in some tumours (Bloomer et al. 1980). This was the first example of the use of receptor-mediated targeting to deliver Auger-emitters selectively to tumours. Importantly, after the labelled Tamoxifen binds to steroid receptors, not only does the drug-receptor complex get internalized, but it binds to DNA, and thus affects receptor-dependent cell kill. Cells without steroid receptors were much less sensitive. This strategy can be thought of as double targeting. Not only was the isotope targeted to tumour cells with estrogen receptors, but once internalized, the second phase of targeting transports the isotope to chromosomal DNA. Unfortunately, the clinical potential of \(^{125}\)I-labelled Tamoxifen and other oestrogen analogues was limited by high uptake in the liver, following intravenous administration (Epperly et al. 1991).

Many of the receptor systems that might be useful for tumour targeting do not have the feature of chromatin targeting, for example in many cases the receptor-ligand complex is degraded after internalization. It was realized that a more generic platform would be required to develop a system for targeting Auger emitters to DNA, especially given that the decay event needs to be located very close to DNA (molecular dimensions) for maximum efficiency of induction of DSB. More generally, the Auger field needed to ‘backfill’ some of the basic science before progressing to the ultimate goal of clinical translation.

**Microdosimetry**

The labelled Tamoxifen strategy was essentially ‘blind’ on key questions such as the relationship between extent of radiobiological damage and the location of the decay event relative to the DNA molecule itself. Moreover this is a multifaceted question, and one aspect required progress in microdosimetry. The first step was the application of Monte Carlo techniques to understand the details of iodine-125 decay (Charlton & Booz 1981); later extended and elaborated (Pomplun et al. 1987). David Charlton was prominent in much of the subsequent advances in the microdosimetry of the Auger effect. Dave is in the group photo of the Oxford meeting in 1987 (Figure 1), but there is a better picture, albeit marking a sad event, in the very nice obituary (Humm & Nikjoo 2013), written by close collaborators (Charlton et al. 1989). The Charlton and Booz paper provided a solid foundation for the future; the number (an average of 21 electrons per decay in the condensed phase, and 13 for the isolated atom) of the emitted electrons and their energies, and the extent of variation amongst the individual decay events.

Now the physics was ahead of the chemistry. Apart from the fact that the DNA was broken by the decay event, there was no information at the molecular level. In the mid-1970s, when the biological significance of the Auger effect was developing, the mainstay in DNA breakage analysis was sedimentation in sucrose gradients, using ultracentrifugation, which yielded the size distribution of DNA molecules, from

![Figure 2](image-url). The beginnings of the radiobiology of the Auger effect. Book cover of the proceedings (with permission) of the meeting in Vienna at which the early observations were discussed. The radioactively labeled counterparts of the deoxynucleosides shown at right (e.g., \(^{3}\)H in the methyl group of thymidine) enabled incorporation of isotopes, notably \(^{3}\)H, \(^{125}\)I and \(^{131}\)I, into DNA.
which the average molecular size was calculated. This was the technology used by Krisch and Sauri in the discovery of the one-to-one relationship between $^{125}I$ decay events in DNA and double-stranded breaks (Krisch & Sauri 1975). But it did not provide information at the molecular level. As is often the case in science, progress awaited the development of new techniques. In the Auger story, the enabling new technologies were first the Wilson cloud chamber, then radiochemical synthesis of labelled nucleosides, and then in the current context, DNA sequencing methodology.

DNA sequencing technology; and more microdosimetry

In 1980 Fred Sanger and Walter Gilbert were jointly awarded half the Nobel Prize for the discovery of DNA sequencing methodology (the other half went to Paul Berg for nucleic acid biochemistry). Fred Sanger was based in Cambridge in England and Gilbert and his co-worker Allan Maxam were at Cambridge, Massachusetts, just across the Charles River from Boston and Harvard Medical School, where Jim Adelstein was based. They used quite different strategies: Sanger relied on DNA polymerase and chain-terminating deoxynucleotides, and Gilbert on ‘cookbook chemistry’ for preferential cleavage at particular bases. There was a debate in Boston at the time as to Allan Maxam’s claim to join in the Nobel Award; he apparently did the painstaking optimization of the reaction conditions for base-dependant chemical cleavage of DNA. Nevertheless, the method subsequently bore the name ‘Maxam-Gilbert DNA sequencing’. Both groups used polyacrylamide gel electrophoresis to separate single strands of DNA according to size/length, at single nucleotide resolution. The key to the method was labelling one end of each DNA strand with $^{32}P$, and locating the separated DNA in autoradiographs. One of us (RFM) visited Fred Sanger in April 1974, on the return trip from an 18-month sabbatical leave at the Karolinska Institute in Stockholm, and recalls Fred proudly showing a 1-metre long gel autoradiograph! Three years later, in 1977, Sanger published the sequence of a bacteriophage DNA of >5000 nucleotides (Sanger et al. 1977). Maxam and Gilbert’s paper came out in the same year (Maxam & Gilbert 1977).

Bill Haseltine was a post doc in Gilbert’s lab in Cambridge (Haseltine et al. 1977) and quickly applied the DNA sequencing technique to analysis of DNA damage by the DNA-cleaving drug bleomycin (D’Andrea & Haseltine 1978). From autoradiographs of DNA sequencing gels analyzing samples of end-labelled full-length DNA molecules, fragmented by either cleavage by bleomycin, or by base specific chemical cleavage, the sites of cleavage within the sequence could be determined.

Later, Bill Haseltine established his own lab at the Dana Farber Cancer Centre at Harvard Medical School, and RFM joined his lab for a 12-month sabbatical during 1979/80. The same method was used to analyze DNA breakage by iodine-125 decay. The results showed that most of the damage is...
focused within a few bp of the site of decay of the iodine atom, for both the $^{125}\text{I}$-containing strand and the opposite strand (Martin & Haseltine 1981).

The experimental data from the Boston experiments is summarized in Figure 5, together with results of simulation experiments described in a landmark paper by Charlton & Humm (1988) that married the $^{125}\text{I}$ microdosimetry with the experiments described in a landmark paper by Charlton & Humm (1988). Reproduced from Charlton DE, Humm JL. 1988. Int J Radiat Biol 53:353–365; with permission.

It is interesting that as well as shedding light on the theoretical basis for the biological effects of Auger decay, this study also contributed to track structure codes then implemented more generally to radiation physics and biology. This model was continually refined and extended by Hooshang Nikjoo, Ekkehard Pomplun (Pomplun 1991), and Michel Terrissol (Terrissol & Pomplun 1994; Kummerle & Pomplun 2005; Edel et al. 2006; Goorley et al. 2008), who were mentored by Dave Charlton. In particular, the crude hemi-annular ‘target’ volume that Charlton and Humm used to represent the nucleotide was refined to atomic volumes (Pomplun 2015), and Hooshang Nikjoo extended the approach from B-DNA to chromatin and nuclear structures (Nikjoo & Girard 2012), enabling development of models for DNA repair (Taleei et al. 2013; Rahmanian et al. 2014; Taleei et al. 2015), and genetic risks (Taleei et al. 2013; Sankaranarayanan & Nikjoo 2015). In the light of these advances, Charlton’s model of representing nucleotides as hemi-annular volumes now seems primitive.

**Molecular fragmentation versus electron irradiation**

At the time when the spectacular radiotoxicity of iodine-125 was emerging from experimental work, it was already appreciated (Figure 6) that two distinct radiochemical events were involved. The central issue is that the multiple electron emission leaves behind, on the daughter Te atom, a corresponding positive charge. The resulting molecular fragmentation was described in an early review (Wexler 1967), which includes the case of methylidioxide cited in the Introduction (Carlson & White 1966) and of methylbromide labelled with $^{80m}\text{Br}$ (Wexler & Anderson 1960). In a more contemporary example, using a model nucleotide system, an average accumulated positive charge of +6 was calculated for the first cascade (Kummerle & Pomplun 2012), which is consistent with the Charlton and Booz average figure of 13 electrons for the isolated atom, given that most decays involve two cascades. A radiation chemist would describe +6 as a very deep hole! Obviously, the question arises: ‘Is the DNA strand breakage a result of this molecular fragmentation (also described by as Coulombic explosion), or is it primarily due to electron irradiation by the Auger electrons that are known to have very high LET at the end of their tracks?’

The question as to the relative contributions of these two mechanisms was addressed first using the DNA sequencing analysis. Lobachevsky and Martin took advantage of advances of technology, in the almost 20 years since the Boston experiments, in particular the availability of synthetic oligodeoxynucleotides, to revisit in more detail the distribution of damage from decay of $^{125}\text{I}$ in a single location. Pavel Lobachevsky’s deconvolution of the data indicated approximately equal contributions for the two mechanisms; molecular fragmentation (non-radiation) and radiation component, which itself comprises two sub classes: Scavengerable (in this case by dimethylsulfoxide [DMSO]) and non-scavengerable (Lobachevsky & Martin 2000).

Much more recently, Igor Panyutin from National Institutes of Health [NIH], Bethesda, MD, USA, has revisited the question of charge migration, using DNA constructs incorporating charge traps, which he reported at the Jülich meeting in 2011 (Ndlebe et al. 2012). Even after all this time, since the early recognition of the question of electron irradiation versus charge fragmentation, for example the paper in 1978 (Hofer et al. 1978), further research is required. Clearly this needs to be incorporated into the microdosimetry calculations – at present it is ignored because there is no theoretical treatment for it.
Targeting Auger damage to DNA with labelled DNA ligands

Returning now to Jim Adelstein’s group in Boston, and the nice idea of receptor-mediated targeting of the Auger effect to tumour, the oestrogen receptor was a good choice because its receptor is translocated to the nucleus. Unfortunately, not all receptor systems that are potentially useful for targeting tumour cells have this bonus of continuation to chromatin. The requirement for a more universal system led to the question of whether DNA-binding drugs could be used as vehicles to take the Auger effect to nuclear DNA. So there was a lot of interest on synthesis and evaluation of $^{125}$I-labelled DNA ligands, and most of this work was done either in Melbourne, Australia, or in the Boston group led by Jim Adelstein and Amin Kassis. There is a nice brief biographical sketch of Amin Kassis in the record of the occasion of him being awarded the Loevinger-Berman Award in 2010 (Howell et al. 2010).

There are two types of DNA ligands: Intercalators which are planar aromatic molecules which fit into the slot between adjacent base pairs in B-DNA, and minor groove binders which are more elongated flexible molecules that follow the helical twist of the minor groove. An extensive review includes images of molecular models illustrating the two modes of binding (Liu et al. 2008). Intercalators were the initial focus (Figure 7), with $^{125}$I-3,6-diamino-4,5-diiodoacridine being the first example (Martin 1977). While this ligand was readily accessible by radioiodination of proflavine (3,6-diaminoacridine), which is commercially available, the structure of the product is ambiguous. Either the mono- or bis-iodination product can be formed, depending on reaction conditions. The subsequent variation solved this problem by using rivanol (6,9-diamino-2-ethoxy-5-acridine) as the iodination substrate. The radioiodinated version was shown to be cytotoxic (Martin et al. 1979), but the relatively low logP restricts cellular uptake. The later Boston designs (Figure 7) are probably better from this standpoint. For example $^{125}$I-3-acetamido-5-iodoproflavine is taken-up and retained in V79 cells and yields a high LET survival curve (Kassis et al. 1989) and is mutagenic (Whaley et al. 1990). Of the two simple iodoacridines, only 2-iodoacridine intercalates, although the $^{125}$I-labelled isomers both induce DNA DSB in plasmid DNA, but the 2-isomer is more efficient (Sahu et al. 1997).

The general experimental approach used for studying $^{125}$I-induced breakage of isolated DNA utilizes the plasmid DNA assay, which nicely distinguishes DNA single-strand breaks (SSB), which relax the tightly coiled parent DNA, from a DSB which produces a linear molecule. These three species are easily separated by agarose gel electrophoresis, and a good approach to data analysis has been developed by Pavel Lobachevsky (Lobachevsky & Martin 2004). In early studies, electron microscopy was used for more qualitative visualization of strand-breakage (Martin 1977).

Minor groove binders (Figure 8) have two main differences compared to intercalators. Firstly the DNA binding affinity is much stronger, and secondly, the minor groove binders have a sequence selectivity; they bind to discrete sites of 3 or 4 consecutive AT base pairs. As a result, when such ligands are labelled with $^{125}$I and allowed to decay in the DNA ligand complex, and the breakage analyzed by DNA sequencing gels, the damage reveals the sites of binding (Martin & Holmes 1983). The first example of a radio-iodinated minor groove binding ligand was prepared by direct iodination of Hoechst 33258 (Martin & Pardee 1985). It is known that substitution of the phenolic group of Hoechst 33258 with an ethoxy group (i.e., Hoechst 33342) markedly improves uptake into live cells (Lydon et al. 1980), so the Boston design (Harapanhalli et al. 1994) is likely to be the better of the two bibenzimidazoles shown in Figure 8, for studies with viable cells or organisms.

![Figure 6. Molecular fragmentation with Auger decay. This early image was designed by one of authors (LEF) in 1968. It illustrates the two distinct damaging components of Auger decay. This Figure, without the legend, was reproduced on the cover of the special issue of the Journal devoted to Proceedings of the Auger Symposium in Jülich, 2011 (see Table 1)](image-url)
In summary, the studies with radioiodinated ligands showed:

- Both intercalators and minor groove binders target $^{125}$I to DNA, and
- Produce DNA DSB with decay, and
- Cytotoxicity and mutagenesis in cell culture experiments
- Location of the $^{125}$I atom in DNA-ligand complex determines efficiency per decay.

This last point warrants further mention. Different designs of the $^{125}$I ligands enables the decay event to be located at different distances from the axis of the DNA helix, and in some cases crystal structures have established the location of the iodine atom in the DNA ligand complex (Lobachevsky et al. 2008), but molecular modelling is also useful. The outcome of all these studies was the discovery of a much steeper than expected relationship between distance and efficiency of double-stranded breakage, compared to the prediction of the Charlton and Humm microdosimetry model. The Boston group also pursued this question, reported at the Jülich Symposium, but the flexibility of some of the ligands compromises interpretation (Balagurumoorthy et al. 2012).

The importance of these studies with labelled DNA ligands is that it provides a generic strategy for targeting of the Auger effect to the DNA of tumour cells. This involves a conjugate with two key components; a peptide that binds to receptors that are over-expressed in tumour cells, and the radioiodinated DNA ligand, joined by a labile linker. The conjugate binds to the receptor, the complex is internalized, and the labelled DNA ligand is released, and finds its way to DNA. This strategy has yet to be exemplified with an Auger emitter, but there is a proof-of-principle using a DNA-binding photosensitizer (Karagiannis et al. 2006).

Clinical examples of receptor-mediated targeting to DNA

Over a period of more than a decade, Reilly and Vallis and co-workers have sought to develop a strategy to target the Auger emitters, principally $^{111}$In, to treat Epidermal Growth Factor Receptor (EGFR)-positive breast cancer, initially using labelled EGF. Encouraging results were obtained in a xenograft model (Chen et al. 2003), and the program has now progressed to a Phase I clinical trial (Vallis et al. 2014). The enhanced toxicity of the labelled EGF for receptor positive tumour cells, compared to normal cells, is attributed to a malfunction of the pathway of cytoplasmic degradation in normal cells, in combination with the (Nuclear Localization Signal) NLS motif in EGF that directs the peptide to the nucleus (Reilly et al. 2006). A $^{99m}$Tc-labelled anti-EGFR antibody conjugate was also investigated, progressing to a Phase I clinical trial (Vallis et al. 2002). This approach was upgraded by using an $^{111}$In-labelled anti-EGFR antibody conjugate modified with an NLS (Costantini et al. 2007).

A concern with the labelled antibody strategy is the question as to whether the decaying Auger-nuclide is close enough to DNA in the ligand-receptor-chromatin complex, given the very stringent distance (or rather, closeness) requirement for efficient induction of DNA DSB upon Auger-decay in the vicinity of DNA (Lobachevsky et al. 2008). Actually, this doubt also extends to the case of the directly labelled ligand. Structural studies show Tamoxifen deeply imbedded in the receptor protein (Brzozowski et al. 1997), which is difficult to reconcile with close proximity to DNA. Nevertheless, the report that the radiotoxicity of a labelled oestrogen analog, $^{125}$I-17-[(125)I]iodovinyl-11$^{13}$O-methoxyestradiol, was similar to that of $^{125}$I-iododeoxyuridine (Yasui et al. 2001), is encouraging, but like earlier such studies (McLaughlin et al. 2006).

![Figure 7. Iodoacridines. The $^{125}$I counterparts of these ligands have been used to investigate DNA damage and cytotoxicity of DNA-associated decay. Upper pair: diiodoproflavine (left) and iodorivanol were the first $^{125}$I-DNA ligands investigated; in Melbourne. The remaining ligands, middle pair: 3-acetamido-5-idoacridine (left) and 3-acetyl-5-idoacridine, and bottom pair: 4-idoacridine (left) and 2-idoacridine were all reported by the Boston group.](image-url)
1981). Even less direct support for high LET damage by \(^{125}\)I-
Johanson1982). This was re-iterated in the presentation of
iodothyronine are difficult to repair (Sundell-Bergman &
the Swedish group at the Auger Symposium in Oxford, as
Figure 8. Iodinated minor-groove binding DNA ligands. The phenolic group in
iodoHoechst 33258 (upper) impedes delivery to intact cells, compared to
iodoHoechst 33342.

1989; Epperly et al. 1991) the simple exponential survival
curve covers a limited range (\(<1\) log of survival). Ironically,
the first of such studies provides the most convincing evi-
dence for high-LET cell killing by a labelled oestrogen analog;
the linear survival curve on a semi-log plot extended for
almost 3-logs of cell kill for \(^{125}\)I-Tamoxifen (Bloomer et al.
1981). Even less direct support for high LET damage by \(^{125}\)I-
labelled receptor ligands comes from the early observation
that DNA DSB induced in receptor-positive cells by \(^{125}\)I-tri-
iodothyronine are difficult to repair (Sundell-Bergman &
Johanson 1982). This was re-iterated in the presentation of
the Swedish group at the Auger Symposium in Oxford, as
also showing that the nuclear uptake of \(^{125}\)I-triiodothyron-
ine was saturable, in contrast to the linear increase in cellular
uptake with the concentration of added ligand (Sundell-
Bergman et al. 1988). The rate of repair of DNA double-strand
breaks, which can now be easily followed by the \(\gamma\)-H2AX
assay, could provide a useful endpoint to identify the more
complex lesions arising from the Auger effect. More generally,
there is a need to reconcile the structural features of the oes-
trogen-chromatin complex with evidence for high LET cell kill,
to be confident that the Auger effect is implicated.

Another general concern of the receptor-mediated strategy
is molecular capacity – can enough Auger nuclide be deliv-
ered and retained by the cell nucleus? In this regard the
innovative approach of the Vallis group to amplify the dam-
age signal by also targeting \(\gamma\)-H2AX as well as nuclear EGF
receptors (Cornelissen et al. 2013) could prove very important.
Similarly, the investigation of the use of gold nanoparticles
to increase the ‘payload’ (Song et al. 2007) is promising.

Clearly, the clinical exploitation of Auger-emitting nuclides
has been elusive. The most widespread example is the use of
\(^{111}\)In-labelled peptides that target the somatostain receptor in
neuroendocrine tumours (Van Essen et al. 2007; Limouris
et al. 2008; Kong et al. 2009). The evidence that the therapy
is mediated by the Auger effect includes the demonstration of
translocation of the labelled peptide to the nucleus (Janson et al.
2000) in patient material, and extraction of the
label with DNA of cultured cells (Hornick et al. 2000).
The same reservations apply as for the EGF case regarding the
stereochemistry of the nuclear complex, and whether the
\(^{111}\)In is close enough to DNA to get the full damage effect of
decay. The fact that endoradiotherapy of neuroendocrine
tumours now seems to have progressed to \(\beta\)-emitters, in par-
ticular \(^{177}\)Lu (Denoyer et al. 2015), suggests that the full
advantage of the Auger effect was not obtained with \(^{111}\)In.
Nevertheless, new opportunities will emerge as further exam-
ples of receptor-mediated targeting to the nucleus are discov-
ered, such as the case of the F3 peptide and nucleolin
(Cornelissen et al. 2012).

**Triplex DNA**

In the receptor-mediated strategy, targeting relies on the ele-
levated expression of tumour-specific cell surface receptors.
A quite different strategy stems from the knowledge that the
genome of tumour cells harbours specific features. The pio-
nieving work starting in the early-1990s of Ronald Neumann
and Igor Panyutin and their colleagues at NIH showed that
labelled Triplex-Forming Oligonucleotides (TFO) provided the
means to target the Auger effect to tumour-specific genomic
sequences (Figure 9).

The triplex DNA story seems to have started in the mid-
1960s with the knowledge that poly(dA) and poly(dT)
can form triple helices (Riley & Maling 1966). The early interest
was to exploit triple helix formation to selectively block gene
expression, for example by photoactivation of a triplex struc-
ture including an oligothymidylicate conjugated to an azido-
phenacyl group (Praseuth et al. 1988), but later the term ‘antigene strategy’ emerged (Helene et al. 1992). Soon after,
the NIH group realized the opportunity to target the Auger
effect, first using a \(^{125}\)I-labelled TFO targeted to a polypurine-
polypyrimidine sequence the \(\text{nef}\) gene of HIV (Panyutin &
Neumann 1994). This paper established that the \(^{125}\)I-labelled
TFO-containing triple helix induced in the unlabeled strands,
the previously established ‘signature’ breakage pattern associ-
ated with \(^{125}\)I decay intimately associated with DNA (Martin &
Holmes 1983), with an overall efficiency of 0.8 DSB per decay.
Subsequently, the terms such as ‘Gene radiotherapy’
(Panyutin & Neumann 1998), ‘Antigene radiotherapy’
(Karamychev et al. 2000; Panyutin et al. 2000; Sedelnikova
et al. 2000) and ‘gene-targeted radiotherapy’ were introduced
(Panyutin & Neumann 2005), and later extended to ‘Anti-
sense radiotherapy’ for targeting to mRNA (Gaidamakova
et al. 2004).

The NIH group (Figure 10) made many contributions to
this field over a period of more than two decades. The early
studies focused on labelled TFO, but later extended to pept-
dide nucleic acids (PNA), especially in the context of targeting
quadruplex structures in the genome, which occur for exam-
ple in BCL2 (Onyshchenko et al. 2009, 2011). The targets
investigated in model systems ranged from repeated sequen-
ces with thousands of copies (Panyutin & Neumann 2005),
through \(\text{mdr1}\) that is amplified in some drug-resistant
tumours (Sedelnikova et al. 2000, 2001; Panyutin et al. 2003;
Panyutin & Neumann 2005) to single copy genes such as
\(\text{HPRT}\) (Panyutin & Neumann 1996, Sedelnikova et al. 1999,
Panyutin et al. 2005). These studies were facilitated by har-
nessing technical developments. An early example being the
incorporation of biotin into the template strand used to syn-
thetize the \(^{125}\)I-TFO (Panyutin & Neumann 1996), enabling
isolation of the $^{125}$I-TFO by removal of the template strand with streptavidin-Dynabeads, after denaturation. This was a great improvement on the laborious, from first-hand knowledge of one of the authors (Martin & Haseltine 1981), method of strand separation in denaturing gels used in the first report from the NIH group (Panyutin & Neumann 1994). Other examples include the gel shift assay to demonstrate binding of $^{125}$I-TFO to target sequences cloned into plasmid, and a Southern blot assay used to demonstrate cleavage by the $^{125}$I-TFO directed to the HPRT (Panyutin & Neumann 1996; Sedelnikova et al. 2002a) and mdr1 (Sedelnikova et al. 2002a) targets.

Following on from the studies in plasmid DNA systems, delivery of $^{125}$I-TFO to the nucleus of cultured cells proved challenging, restricting investigations to isolated nuclei or digitonin-permeabilized cells (Panyutin et al. 2000, 2003, Sedelnikova et al. 2000). Nevertheless, the demonstration of almost 80% efficiency of mutagenesis, albeit in a prokaryote system (Panyutin et al. 2000), illustrated the potential of anti-gene radiotherapy. Delivery using a cationic liposome system, could be improved by using a nonspecific oligonucleotide as ‘ballast’ or by conjugation of TFO to a nuclear localizing peptide sequence (NLS), enabling delivery to nuclei of intact cells (Sedelnikova et al. 2002b; Panyutin et al. 2003). Radiotoxicity was initially demonstrated by the clonogenic survival endpoint (Sedelnikova et al. 2000, 2004), but gamma-H2AX proved more convenient (Sedelnikova et al. 2004; Panyutin & Neumann 2005), at around the time when the one focus = one DSB approximation was established (Sedelnikova et al. 2002b). However, the absolute efficiency of delivery was still disappointing, with apparently only about 0.1% of decays in $^{125}$I-TFO occurring in DNA-bound vehicle (Sedelnikova et al. 2004).

Dahmen and Kriehuber revisited the $^{125}$I-TFO antigen radiotherapy concept and reported their findings at the Jülich symposium (Dahmen & Kriehuber 2012). They confirmed the key findings of the NIH group, except for a 40-fold higher efficiency of cell kill ($D_{37}$/decays/cell), which they attribute to improved efficiency of their transfection system which involved electroporation. Thus, delivery of labelled TFO remains a challenge, even for intact cultured cells, let alone in vivo. Accordingly, Dahmen and Kriehuber concluded that targeting $^{125}$I-TFO to single genes as a useful research tool. Meanwhile, the therapeutic potential of Auger-mediated anti-gene radiotherapy awaits a technological advance in TFO delivery.

**K-edge ionization – photon activation therapy (PAT)**

Radiosensitization by incorporation of bromo-and iodo-deoxyuridine was established in the 1960s, initially by Szybalski and co-workers (Erikson & Szybalski 1963), but the specific idea to exploit the Auger cascade from K-edge ionization was first published by Tisljar-Lentilius, in a Jülich-Brookhaven collaboration (Tisljar-Lentilius et al. 1973). The term ‘photon activation therapy’ (PAT) was introduced by Ralph Fairchild et al. in 1982, in anticipation of the Brookhaven synchrotron in 1984 (Fairchild et al. 1982). Fairchild et al. clearly distinguished between the ‘biological’ radiosensitization, independent of photon energy, and the further enhancement from Auger electrons derived from K-edge activation. This distinction is illustrated in Figure 11, which is adapted from a paper presented at the Oxford symposium (Maezawa et al. 1988). Fairchild et al. described iodine, compared to bromine, as the ‘only viable choice’. It is interesting to note (Figure 1) that Ralph Fairchild attended the Auger symposium in Oxford in 1987 (but curiously, did not present a paper), at which Humm and Charlton presented calculations of simulated DNA DSB yields for K-edge irradiation of DNA with incorporated bromine, and concluded that the Auger cascades were ‘relatively unimportant’ (Humm & Charlton 1988). Later calculations indicated a significant effect for iodine (Karnas et al. 2001, Moiseenko et al. 2002).

Clearly, the advent of intense synchrotron photons that could be precisely tuned to energies above and below the K-edge of target atoms provided the opportunity to investigate and develop PAT. Meanwhile, while awaiting the completion of the Brookhaven facility, in the 1982 paper, Fairchild et al. proposed an ingenious combination of PAT and brachytherapy, choosing isotopes with gamma emissions with energies suitable for excitation of iodine (Fairchild et al. 1982). Samarium-145 was identified as an ideal isotope for brachytherapy combined with infusion of iododeoxyuridine, and this idea was subsequently elaborated (Fairchild & Bond 1984, Goodman et al. 1990). Much later, the palladium-103/Pt combination was suggested (Laster et al. 2009), and further combinations have been considered (Bakhshabadi et al. 2013). However, this concept does not seem to have been followed.
up clinically, with most attention focused on synchrotron sources.

Following investigation of iododeoxyuridine PAT, comparing megavoltage and 100 keV X-rays, which reported modest (15%) enhancement for PAT (Miller et al. 1987), the first results from Brookhaven, using photons above and below the iodine k-edge, reported an enhancement of 1.4, on top of the radiosensitization factor of 2.2 (Laster et al. 1993). Similar cell culture studies at the Grenoble Synchrotron reported PAT with iododeoxyuridine-treated cells (Corde et al. 2004). This was later followed by a study in a rat glioma model, but no enhancement was observed (Rousseau et al. 2009). The Corde et al. study also reported sensitization by iodine contrast agent, which resulted in low LET type survival curves, whereas PAT was associated with exponential survival curves, clearly distinguishing PAT from sensitization. Unfortunately, in the more recent literature, the term PAT has been confused with sensitization, and used in conjunction with studies with iodine contrast agent and heavy metal nanoparticles, which are unlikely to be close enough to DNA to produce Auger damage to DNA upon activation. Nevertheless, dose enhancement (albeit not PAT according to the original definition by Fairchild) is a potential strategy to improve therapeutic ratio given the possibility to exploit the blood brain barrier to enable preferential delivery of contrast agent, for example, to brain tumours.

The widespread use of platinum drugs in cancer chemotherapy, often in conjunction with radiotherapy, has focused attention on the potential of PAT, with the added benefit of somewhat better photon penetration associated with higher k-edge energy. The potential was established in early studies at the Photon Factory in Japan (Le Sech et al. 2000, Kobayashi et al. 2002) and promising results have been obtained with the rat glioma model in Grenoble, for both CisPt (Biston et al. 2004) and carboplatin (Rousseau et al. 2007). A subsequent study with carboplatin compared 6 MeV X-rays with 78.8 keV synchrotron photons and found no difference in efficacy, from which the authors concluded that the effect was ‘not due to the production of Auger electrons and photoelectrons emitted from the Pt atoms’ (Bobyk et al. 2012).

Another recent study with glioma-bearing rats administered thallium and irradiated with 50 keV photons found an insignificant increase in survival for the combination, compared to radiation-only (Ceberg et al. 2012). In the introduction, the authors pointed out that most of the metal is taken-up into the cytosol. It is interesting to reflect on the work of Apelgot, who noted at the Oxford Auger Symposium (Apelgot & Guille 1988) that metals such as Cu and Zn seem to be intimately associated with DNA, and inclusion of the Auger emitter $^{64}$CuCl$_2$ in the medium for prolonged periods (> 24 h) resulted in exchange and decay-induced lethal damage to DNA close-up. [Curiously, the same high LET-type (simple exponential) survival curves were obtained for $^{67}$Cu, which is not an Auger emitter (Apelgot et al. 1989)]. However, it seems likely that the amounts of such heavy metals naturally bound to DNA would not be sufficient for PAT, nor provide a differential between tumours and normal tissues.

In conclusion, it is sobering to reflect, that in spite of availability of synchrotron irradiation for more than 30 years, clinical PAT has not advanced beyond the Phase I-II clinical trial of iododeoxyuridine in combination with RT of anaplastic astrocytoma, which claimed a modest survival benefit relative to historical controls (Urtasun et al. 1996); a claim that was
disputed in an editorial comment (Phillips 1996). In any case, these studies used conventional (high energy) photons, so PAT does not correctly describe the modality.

**Gadolinium Neutron Capture Therapy (GdNCT)**

Boron Neutron Capture Therapy (BNCT) is a well-established concept for cancer radiotherapy, the crux of which is the following nuclear equation:

\[
{^{10}\text{B}} + n \rightarrow ^{4}\text{He} + ^{7}\text{Li} + 2.3\text{ MeV}
\]  

(1)

describing the fission reaction that results from capture of a low energy thermal neutron by a 
\(^{10}\text{B}\) nucleus. The products are very high LET particles: An alpha particle and \(^{7}\text{Li}\) nucleus. Compared to the nuclei of biological elements, the \(^{10}\text{B}\) nucleus has an exceptionally reactivity. This reactivity is quantified by the thermal neutron capture cross section, which for \(^{10}\text{B}\) is about 4000 barns (compared to 0.0002, 0.004 and 0.3 for O, C and H, respectively). The concept of BNCT is to use a \(^{10}\text{B}\)-drug that accumulates in tumours, and irradiate with thermal neutrons from a nuclear reactor, and the tumour is selectively damaged by the fission particles.

As outlined in a recent review (Barth et al. 2005), most clinical experience has been in Japan, and initially, many of these treatments were done at the Kyoto University Research Reactor by the neurosurgeon Hiroshi Hatanaka. He treated >120 brain tumour patients between 1968 and when he tragically died in 1994 at the age of 62, but the claim of better outcomes compared to conventional treatment is controversial.

One of the authors (RFM) was invited to join the Australian contingent of a BNCT collaboration between Australia and Japan during the 1980s. The common interest was the high LET feature shared by Auger electrons and the products of BNC. From the necessary background reading around BNCT, the huge thermal neutron capture cross section the gadolinium-157 (also a non-radioactive isotope) was striking: 242,000 barns! The Australian contingent was headed by Barry Allen, a nuclear physicist at the Australian Nuclear Science and Technology Organisation (ANSTO) that manages Australia’s nuclear reactor in Sydney. At first, Barry was not enthusiastic about the potential of the Gadolinium Neutron Capture (GdNC) reaction:

\[
{^{157}\text{Gd}} + n \rightarrow ^{158}\text{Gd} + 
\gamma + 7.94\text{ MeV}
\]  

(2)

because it does not yield high LET particles with a restricted range from the site of reaction, rather it produces high energy gammas. However by analogy with the \(^{125}\text{I}\) decay scheme, it seemed possible that some of the gamma energy would yield conversion electrons, and thus Auger electrons. This hypothesis led to an experiment done with a very nice thermal neutron beam developed by Barry Allen in the small experimental reactor (MOATA) then at ANSTO. The experiment involved combining plasmid DNA with gadolinium cations, plus and minus the metal chelator, ethylenediaminetetraacetic acid (EDTA). Without EDTA, the \(^{157}\text{Gd}\) cations bound to the DNA polyanion, but they were sequestered away from the DNA with the addition of EDTA. After irradiation for several hours, linear plasmid DNA was produced in the non-EDTA sample, but not in the EDTA-containing control (Martin et al. 1988). This proof-of-principle experiment demonstrated that DNA double-stranded breaks are produced from GdNC on DNA, attributable to the Auger effect.

Translating this to even just cell culture experiments required a \(^{157}\text{Gd}\)-labelled DNA binding ligand. Minor groove binding ligands were synthesized (Martin et al. 1992), but failed to produce DNA DSB when mixed with plasmid DNA and exposed in the thermal neutron beam. This can be attributed to the fact that the chelation cage cannot be accommodated in the minor groove, so the \(^{157}\text{Gd}\) atom is simply not close enough to the DNA. Nevertheless, there is continuing interest in GdNC, but until the \(^{157}\text{Gd}\) is targeted to the DNA molecule, GdNC will merely be dose-enhancement with the main feature, the Auger effect, not exploited. Maybe it is possible to design a DNA-binding molecule with the chelating cage, and that fits in the major groove. Such a ligand could also be useful for PAT.

**Discussion and Conclusions**

Translation of the features of the Auger effect to cancer radiotherapy has been elusive. The only clear example is photo-activation after infusion with bromodeoxyuridine or iododeoxyuridine, for which there were clinical trials for anaplastic astrocytoma in the 1980s, but without clear benefits. These trials involved megavoltage radiotherapy, so the contribution of photo-activation was probably minor. Synchrotron irradiation with lower energy photons would enhance the Auger contribution, but after initial preclinical studies from the Grenoble facility, there seems to be no intention to progress to clinical studies with iododeoxyuridine. Similarly, the Fairchild idea of PAT brachytherapy using appropriate gamma sources does not seem to have been
taken up. The clinical photosensitization associated with infusion of halopyrimidines may be a major factor, as well as the difficulty in achieving sufficient replacement of thymidine with the halopyrimidine.

The use of 111In-labelled octreotide to target somatostatin receptors in neuroendocrine tumours was reported to exploit the Auger effect, but this unlikely given that β-emitters particularly 177Lu now supercedes 111In in that setting. However, it is interesting that this example of clinical Auger radiotherapy stems from the pioneering studies of Bill Bloomer and Jim Adelstein with 125I-Tamoxifen in the 1980s, which introduced the use of receptor-mediated targeting systems in which the ligand receptor complex is translocated to the nucleus.

The work of Vallis and Reilly on targeting via the EGF receptor, which is clearly progressing to clinical studies, is also in this category, and it is particularly interesting that nuclear translocation is possibly specific to breast tumour cells. Nevertheless, there is a question that hovers over the nuclear receptor approach generally, namely the extent to which the Auger effect contributes. We now know there is a very steep distance-dependence between the distance of the decaying Auger nuclide from DNA and the efficiency of double-strand break induction. Precise structural information is obviously required, but it seems unlikely that the decaying nuclide in the nuclear complex would be close enough to take full advantage of the Auger effect. This hurdle (which incidentally, is already cleared for triplex targeted Auger anti-gene radiotherapy concept) could possibly be overcome by using ‘validated’ labelled DNA ligands in conjugates delivered by a receptor-mediated process, but this potential has not yet advanced to clinical studies. Furthermore, the prospect of success is limited by the capacity (i.e., nuclides deliverable per nucleus) of receptor-mediated systems. In this context, the use of nanoparticles to amplify the radionuclide load per cell (for example, Song et al. 2016) could be important.

The ‘traffic volume’ issue is even more critical for PAT. Whereas carrier-free nuclides are accessible for Auger endoradiotherapy, PAT is constrained by the reality of photon cross-sections (or neutron capture cross-section in the case of GdNCT). On the other hand, compared to Auger endoradiotherapy, for which off-target delivery to normal tissues is always going to be a limiting factor, the binary nature of PAT is a great advantage. It is feasible that small molecule DNA ligands that are delivered directly, rather than by receptor-mediated endocytosis, could deliver a much larger number of photo-activatable atoms (e.g., iodine, heavy metals) to DNA for efficient photo activation, but still under the toxicity ‘ceiling’. This approach might also be open for GdNCT.

In summary, there are many obstacles for translation of the Auger effect to the clinical reality of cancer radiotherapy, but it is still possible that these hurdles can be overcome by diligence and thoughtful optimism.

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The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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