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Chapter 16

Natural Compounds, Antioxidant and Antiandrogens in the Prevention of Prostate Cancer: In vivo Evidences from Murine Models and Human Clinical Studies

Rossano Lattanzio, Alessia Lamolinara, Mauro Piantelli and Manuela Iezzi

Additional information is available at the end of the chapter

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1. Introduction

Prostate cancer (PCa) is the most frequent malignant neoplasia in men. The number of cases has continuously increased over the past decades, partly due to the higher life expectancy. Additional factors are the high caloric diet and lack of physical exercise, typically seen in the Western countries. Notably, up to 40% of cancer incidents are preventable by consuming a healthy diet, regular physical activity, and maintenance of optimum body weight, and more than 20% by consuming vegetables and fruits. PCa represents an ideal candidate disease for chemoprevention. It is typically diagnosed in elderly men and even a modest delay in the neoplastic development could result in substantial reduction in the incidence of the clinically detectable disease. In this chapter we will review the history, the development, and the applications of some of the most common animal models of PCa, and we will discuss the role of animal models in translational research.

2. Body

Prostate cancer (PCa) is the most common non-cutaneous malignant neoplasm in men in Western countries, responsible for the deaths of approximately 30,000 and 85,000 men per year in the United States and Europe, respectively [1,2]. The number of cases is increasing rapidly in step with the growing number of men >50 worldwide, strategies for the prevention of PCa and its progression are urgently required. Since studies of chemo-
preventive agents in humans are hampered by the long latency period and challenging epidemiological problems, reliable preclinical models can be useful to overcome these problems. Early prostate tumorigenesis is apparently characterised by dysplasia that starts with proliferative inflammatory atrophy as the prelude to low-grade Prostatic Intraepithelial Neoplasia (PIN), high-grade PIN, primary cancer, metastatic cancer, and hormone-refractory cancer. During this progression, genetic damage accumulates within cancer cells [3,4]. Animal modelling has made a significant contribution to the study of prostate development and disease. Identification of the molecular features of PCa pathogenesis and progression could be greatly facilitated by laboratory and clinical models. However, a prerequisite for the elaboration of useful models is a better understanding of the molecular characteristics of human PCa. This puzzle, in addition to the well-known inter- and intra-individual heterogeneity of the disease itself and its multi-faceted nature, has necessitated the development of several complementary model systems. The most effective animal models will be those that most closely mimic the phenotypic and genetic changes accompanying the progression of the human disease. Systems shown to be promising include the dog, the rat, the human xenograft, and the genetically manipulated mouse. They have been widely employed to test preventive regimens, combinations of chemopreventive agents and/or drugs, cancer vaccines, and targeted treatments [5-12]. This paper reviews the history, development, and applications of some of the most common animal models, and discusses their pros and cons in translational research.

3. Canine models

The dog is the animal known to commonly develop high-grade PIN and PCa spontaneously in a human-like manner [13]. The many similarities between the canine and the human form include the morphologic and phenotypic heterogeneity of the tumoral lesions, the age-dependency of tumor occurrence, and the propensity to metastasize to bones in an osteoblastic manner [14,15]. Androgen-dependency, on the other hand, is ruled out by a similar incidence in castrated animals [15], while a relatively long latency, the low incidence of spontaneous disease, the impracticability of genetic manipulation, and the high expense of maintaining dog colonies [16,17] are other limitations of canine systems.

4. Rat models

Spontaneous PCa is sometimes observed in some strains of rats [18]. The Dunning model [19] is the most popular. The original R-3327 tumor arose spontaneously in an inbred Copenhagen rat, and was translated into a syngenic Copenhagen x Fisher F1 rat. It is a slow growing, well differentiated and non-metastatic form. Several sublines with different characteristics mimicking some aspects of the human disease have since been developed [20-23]. Copenhagen and Wistar rats also develop a wide range of PCa phenotypes [24,25]. This variability, however, coupled with the rarity and long latency of these tumors, and their lack of
metastases, bar the realistic employment of such models [12], though the recent elaboration of knockout methods [26-28] indicates that greater use could be made of genetically engineered rats in the future [29].

5. Xenograft models

In immunodeficient nude mice tumors grow after injection of cancer cells or xenograft implantation with no evidence of a graft-versus-host response. In function of the number of cells injected, or the size of the xenograft, the tumor will develop over 1–8 weeks, 1–4 months, or longer, and its response to treatment can be studied [30]. By comparison with in vitro studies, this approach offers several advantages, especially a 3D structure complete with tumor-induced angiogenesis, hormonal, paracrine/autocrine factors, and metastasis [12]. Xenografting of human PCa began in the 1970s [31]. Thereafter several cell lines that displayed different PCa phenotypes when injected into athymic nude mice have been developed [32,33]. This model has been used to show the ability of tumor xenografts to metastasize to the lymph node and bone, the two most common human sites [34].

Mice with an autosomal recessive Severe Combined Immuno Deficiency mutation (SCID mice) were identified in 1983 [35]. This mutation results in a lack of T- and B-lymphocyte function. However, normal natural killer (NK) cells and myeloid function are present, and in some SCID mice, some B and T cells are still present [36]. In this model subcutaneous injection of HER2/neu overexpressing human CLNCaP cells has shown that HER2/neu induces androgen-independent tumor growth through modulation of the androgen receptor signalling pathway[37].

In 1995, the features of this model were improved by crossing SCID mice with nonobese diabetic (NOD) mice, which lack in NK cells, antigen-presenting cells, and circulating complement [38]. NOD-SCID mice accepted foreign tissue more successfully and were more immunodeficient than SCID mice. This strain has been used to elaborate a model for orthotopic implantation of PC-3 and DU145 cells with a tumor take efficacy of >80% for both lines [39]. Some xenograft models result in metastasis to bone after intracardiac injection of bone cells that probably survive in a niche whose microenvironment is optimal for their seeding and growth. However intracardiac injection is not an ideal procedure and attention has thus been focused on xenografts to orthotopic sites such as the prostate. The success rates depend on the host strain and the use of hormones or Matrigel to provide adequate growth factors and a scaffold for cell growth [40-42].

The immunodeficiency mouse model has been further improved by crossing NOD-SCID mice with interleukin-2 receptor gamma null mice (NOG/NSG mice). These long-living mice (median 90 weeks) totally lack B, T, and NK cell activities, and cytokine signaling, together with no age-related “leakiness”. They have a higher xenograft success rate and are more effective than other models, particularly in long-term studies involving prostate and non-prostate cancer cells [43-45].
For preclinical prostate studies, most laboratories employ human PCa cell lines xenografted in mice. Many excellent reviews of the characteristics of these lines have been published [46-50]. The most widely used, each with thousands of studies published according to PubMed, are the classic three lines PC-3, LNCaP, and DU145, while each of the other lines has less than 200 citations [8]. These cell lines do not represent the steps of PCa progression. For example, almost all cell lines, including the most popular, were obtained from metastatic deposits: PC-3 from bone, LNCaP from lymph node, and DU145 from dural metastasis. In addition, PC-3 and DU145 are androgen receptor (AR) negative and LNCaP expresses a mutated AR. Again, cell lines, and their sublines in particular, are not fully genetically, functionally and phenotypically characterized, nor is there a method for standardization [8,46-48].

6. Transgenic mouse models

The last ten years have witnessed a remarkable shift in animal-based cancer research from xenografted tumor to transgenic models since it is believed that they will recapitulate the complete course of carcinogenesis more accurately[48]. This assumption stems from the recognition of several advantages that transgenic models offer when compared to xenograft systems. Among these are that the process of carcinogenesis begins with normal cells, progresses through distinct genetic and histological stages, occurs in an immuno-competent host and in its own cellular microenvironment, and that metastasis can occur along routes and to sites relevant to the clinical disease. A perhaps unrecognized attribute lies in the fact that, because the disease is not initiated by human action but by a genetic program that passes through the germline, the disease process is “reset” each generation. Statistically, the progression of a transgenic model of cancer should therefore be precisely recapitulated across time and between colonies. Given appropriate record keeping and data analysis, this feature should allow epidemiological-style investigations of great statistical power, free from both the mathematical noise of genetic and environmental variation, and from many of the economic and ethical constraints of human medicine.

Genetically engineered mouse (GEM) models have been utilized to identify pathways involved in carcinogenesis and investigate the role of particular gene mutations/deletions, and validate key genes as therapeutic targets. These models have been widely employed to test preventive regimens, combinations of chemopreventive agents and/or drugs, cancer vaccines, and targeted PCa treatments [5-12]. To mimic the human disease, GEMs could be generated through several mechanisms, such as overexpression or activation of oncogenes, elimination of target suppressor genes (Knock-outs), or generating dominant negative proteins that disrupt the function of regulatory genes.

The methods initially reported for genetic mouse modification involved the introduction of DNA constructs designed to induce the expression of proteins under the control of strong tissue-specific promoters, such as probasin and PSA. Simian virus 40 (SV40) large T antigens (Tag) were widely used because of their transforming ability. They interact with and sup-
press the tumor suppressor protein p53 and retinoblastoma [51,52]. In addition, the small t antigen interacts with the serine/threonine-specific protein phosphatase 2α to induce transformation [53].

The first model involving the expression of SV40 tumor antigens to develop PCa in the mouse was the C3(1)-Tag model [54]. Targeting the Tag expression to the prostate was achieved by using a region of the C3 (1) gene, the rat prostatic steroid binding protein gene. Most C3(1)-Tag mice developed PIN after about eight weeks of age. Invasive adenocarcinomas followed after 28 weeks in about 40%. These tumors rarely metastasized (<4%), and always to the lungs. However, SV40 expression was also detected in the mammary and salivary gland, while all females develop mammary intraepithelial neoplasia that may progress to mammary carcinomas [55]. More effective prostate targeting was obtained in later models. Relatively few studies have used the C3 (1)/Tag model.

The transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse [56,57] is the best known and most widely used PCa model because it closely mimics the human disease. In this model, expression of both large and small SV40 early genes (T and t antigen, Tag) are driven by the prostate-specific promoter probasin that leads to cell transformation within the prostate. In this model, Tag are under the control of the minimal rat probasin –426/+C28 fragment. All male TRAMP mice develop PCa spontaneously: as in humans, they develop PIN, and well- or moderately-differentiated adenocarcinomas (between 10 and 20 weeks of age) and undifferentiated carcinomas (expressing or not AR) as well as phyllode tumors in the seminal vesicles [58,59]. Most adenocarcinomas arose in the dorsolateral lobe, which is considered most analogous to the peripheral zone where the human disease originates [10]. TRAMP was the first mouse model to display distant organ metastases, albeit rarely to the skeleton. Metastatic progression can be observed after 28 weeks of age, when almost all mice display lymphatic and >60% lung metastases from AR-, poorly differentiated (PD) tumors that constitute the main “lethal phenotype” in the TRAMP mouse on account of their fast growth and consequent acute renal damage due to compression, and also because they are the source of distant metastases and systemic cachexia [60]. These phenomena can also occur in the absence of other physiologic sequelae of metastatic disease [61]. An issue with the TRAMP model is that its most frequent lethal and metastatic malignancy (i.e. the PD tumor) has been reported to be of neuroendocrine nature and origin, while the simultaneous loss of p53 and Rb could increase susceptibility to neuroendocrine cancer [62-64].

The TRAMP mouse has become a popular preclinical model for studying chemoprevention/treatment of PCa, and elucidation of the antitumorigenic effects of many classes of chemopreventive/therapeutic regimens, including anti-androgen, anti-estrogen, anti-angiogenic, ornithine decarboxylase inhibitors, green tea polyphenols, COX-2 inhibitors, phytoestrogens, retinoic acid, grape seed extract, flavonolignans, etc (Table 1). This model enables comparison of the efficacy of treatments. A significant decrease of incidence and a delay of tumor progression was observed following anti angiogenic treatment (endostatin and angiostatin gene therapy), and lycopene and tomato supplementation. Other promising antioxidant agents include green tea, soy, resveratrol, crucifers, curcumin, tocotrienols, triterpenoids and methyl-selenium.
<table>
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Table 1. Preventive/Therapeutic Regimens Tested in the TRAMP Model of Prostate Cancer
To increase the transgene expression beyond that obtained with the minima probasin pro-
moter, as in the TRAMP mouse, an 11.5 kb 5’ flanking fragment of the prostate-specific probas-
bin promoter (large probasin) has since been isolated [65], and used to direct large T-
antigen expression to the dorsolateral and ventral prostate (Lady mouse model). The second key difference in this model is that the large probasin promoter was linked to a deletion mu-
tant of the SV40 T-antigen that expressed only the large T-antigen [66,67]. The Lady model is
advantageous because expression is high, but the PCa progression is less aggressive, begin-
ning with low to high-grade PIN and proceeding to carcinoma with neuroendocrine fea-
tures. However, metastatic progression was not seen [5,67]. Several other transgenic mouse
models have been developed with or without the involvement of SV40 antigens and with
different strategies (reviewed in ref. [12]). In summary, while T antigen expression generally
induces castration-resistant, aggressive and metastatic PCas, often with a neuroendocrine
phenotype, the specific expression of other oncogenes in the prostate results in a mild phe-
notype that rarely progresses to adenocarcinoma.

7. Knockout mice

7.1. Whole body models

The roles of genes significant in prostate carcinogenesis can also be studied in, whole-body
knockout models. Here, however, the gene involved is knocked out ubiquitously, and its
specific role in a given organ cannot be readily determined. Estrogen receptor b knockout
mice display hyperplastic foci in the prostate or even no pathological changes [68]. Deletion
of retinoic acid receptor γ determines squamous metaplasia of prostate and seminal vesicles,
but not carcinomas [69]. p27 knockout mouse display prostatic hyperplasia histologically
similar to that observed in human BPH, but not PIN, and a pathogenetic role of p27 loss in
BPH development in both mice and humans has been suggested [70]. Inactivation of T
(phosphatase and tensin homolog deleted on chromosome 10) prevents activation of AKT
and apoptosis resulting in embryonic lethality. However, haploinsufficiency leads to early
stages (PIN) of prostatic carcinogenesis [71]. Double-knockout models in which loss of
PTEN is associated with loss of other tumor suppressors (p27, Nkx3.1, and p53), are charac-
terized by more aggressive tumor phenotype. The highest stage of tumor progression was
adenocarcinoma (PTEN x p27 mouse) [72], lymph node metastases (PTEN x Nkx3.1 mouse)
[73], and high grade PIN (PTEN x p53 mouse) [74]. In addition, several mouse models with
up to 5 genetic hits demonstrated, as expected, the complexity of the events required for a
complete progression of prostatic tumors from low-grade PIN to metastatic disease (see re-
view [75]).

7.2. Conditional models

The “old” (1979) [76] Cre-loxP system was used to produce mice with prostate-specific alter-
atations. Cre is a recombinase that promotes specific genetic recombination in trans at loxP
sites. The Cre-loxP system was developed and used for genetic recombination first in yeast
and later in mice [77,78]. Many genes knocked out with the whole body strategy were also knocked out by using a conditional approach that results in higher prostate tumor severity. As an example, tissue-specific deletion indicated that homozygous loss of prostatic PTEN led to most stages of prostate tumor progression (metastatic disease) when compared to whole-body haploinsufficiency, where only PIN was present [79]. At present, the Cre-lox system is diffusely employed to generate mouse models characterized by cell-type-specific and tissue-specific genetic modification (see recent review in ref. [12]). The probasin and the prostate specific antigen (PSA) promoters were extensively utilized to induce targeted Cre expression in the prostate. PB-Cre and PSA-Cre mice have been employed to delete the intraprostatic expression of PTEN, Rb, p53, APC, IGF1 and PTEN, Nkx3, respectively.

E-Resources for mouse models of human cancer, including PCa, are also available online (http://emice.nci.nih.gov/,http://cancermodels.nci.nih.gov/,andhttp://cancerimageresources.nci.nih.gov/).

8. Clinical trials

Mouse models have significantly contributed to our understanding of PCa biology through their identification of new cancer genes and biomarkers, and their illustration of the molecular and cellular mechanisms underlying tumor initiation and progression. They have also been employed in a preclinical setting to test novel preventive and/or therapeutic strategies [5,6,8-12,80]. Mice, in fact, offer several advantages. They are small, relatively inexpensive, and reproduce rapidly with large litters. More importantly, technical advances have facilitated the generation of defined genetic modifications that can also be spatially controlled, to mimic human prostate carcinogenesis. In general, and perhaps not surprisingly, a variety of phenotypes are obtained depending on the specific genetically engineered mouse model, but none exactly mimics the human disease. Although preclinical studies and the epidemiological evidence suggest that specific dietary components or nutritional supplements influence overall mortality and/or reduce the risk of PCa, randomized, controlled clinical trials provide high-quality evidence of benefit, no effect, or even harm. Examples of ongoing clinical trials are reported in Table 2. In the last ten years, several primary prevention trials have been reported (reviewed in ref. [11,81]). Preventive strategies in a clinical setting have focused on two approaches: antioxidant regimens to reduce DNA damage and suppression of androgenic stimulation [82]. Since a wealth of preclinical and epidemiologic data indicated that selenium and vitamin E reduce PCa, these compounds were evaluated in humans. The Nutritional Prevention of Cancer (NPC) trial found a 63% reduction of PCa incidence (secondary endpoint) following the administration of selenized yeast [83]. The Alpha-Tocopherol Beta-Carotene Cancer prevention study (ATBC), one of the first large studies (14,569 subjects enrolled), investigated the prevention of lung cancer among male smokers. The results indicated that beta carotene supplements increased the risk of lung cancer, rather than preventing it, and that vitamin E had no effect [84-86]. However, a significantly lower risk of PCa was observed for participants receiving vitamin E alone. The NPC and ATBC findings...
underpinned the NCI-sponsored selenium and vitamin E cancer prevention trial (SELECT). This randomized 35,533 men into four groups: (1) selenium/placebo, (2) vitamin E/placebo, (3) both agents, and (4) placebo alone [87]. At a mean of 5.5 years neither agent reduced risk of PCa. However, at a mean of 7 years and with an additional person-year of follow-up, men receiving vitamin E alone had a significantly increased risk of PCa (Hazard Ratio 1.17, 99% CI 1.004–1.36, \( P = 0.008 \)) [88]. Does vitamin E prevent or promote cancer? More research on the biological activities of the forms and mixtures of tocopherols (alpha, gamma, and delta), and their baseline serum levels should be considered (analyses and discussion in ref. [81,89,90]).

The most promising agents for preventing PCa are probably the 5-alpha reductase inhibitors (5-ARIs). Five-alpha reductase catalyzes the conversion of testosterone to the more active dihydrotestosterone. The Prostate Cancer Prevention Trial (PCPT) and the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) Trial evaluated the activities of two 5-ARIs, finasteride and dutasteride, respectively (reviewed in ref. [81,91]). 5-ARI use for 4-7 years reduced the overall risk of biopsy-detectable PCa by 23-25%. All the prevented cases are either low-grade (PCPT) or GS \( \leq 3 + 4 = 7 \) prostatic carcinoma (REDUCE). It is unclear whether the slightly increased risk of high-grade cancers in both trials is real or an artifact. In addition to the risk of androgen-independent tumors, the side effects of 5-ARI such as neurodegeneration, osteoporosis, cardiovascular diseases, genitourinary dysfunctions, and hormonal disarrangement limit their use as primary chemopreventive drugs [92-94].

Clinical translation has thus proved to be a general failure when viewed against the optimism aroused by preventive treatments (antioxidant, anti-hormonal, anti-inflammatory, anti-angiogenic etc agents) in the preclinical setting. It has been proposed that species-specific differences, and differences in time of treatment intervention age, trial design enrolment criteria, genetic variation, and the choice, dose, and bioavailability of preventive/therapeutic agents are lie behind for the discrepancy [11]. The most substantial challenge posed by mouse models of PCa, as for other tumors, is their species-specific differences. The lifespan of a mouse is 25-50 times shorter than that of humans, and mice are 3000 times smaller, with consequent differences in pharmacokinetics [95,96]. Anatomically, the human prostate is a single alobular organ with a central, a transitional, and a peripheral zone, whereas the murine prostate comprises four paired lobes located around the urethra, namely the anterior (or coagulating gland), dorsal, lateral, and ventral prostate. The dorsal and lateral lobes are treated as one (the dorsolateral lobe) as they share a ductal system. This lobe has been described as the most similar to the human peripheral zone where most carcinomas arise [97,98]. According to the Bar Harbor Pathology Panel consensus opinion, however, there is no direct relationship between any mouse lobe and any of the human zones [58]. Histologically, the mouse and the human prostate display similar cell types (secretory, basal and neuroendocrine), but their ratio varies from one species to another [99,100]. Mice have fewer basal cells and a discontinuous layer on the basal membrane, whereas in humans, this layer is continuous between secretory cells and the basal membrane. Neuroendocrine cells, rare in humans, are even more rare in mice. The human prostate is characterized by an abundant fibromuscular stoma, whereas the murine gland has a small stromal component. Mice are
susceptible to malignancies. By comparison with humans, however, they tend to have more sarcomas and lymphomas and very few epithelial tumors, probably due to differences in relative telomere activity [101-103]. Telomerase, mostly inactive in cells from adult humans, is present in mouse cells, which can thus be transformed/immortalized more easily than their human counterparts, and fewer genetic hits are required to bring about neoplastic transformation in mice than in men. Inactivation of telomerase in the mouse model may be necessary to more accurately recapitulate human cancer phenotypes [80,104].

Most primary PCa prevention studies used mice with an average age of 4-8 weeks, by which time they are considered to have attained sexual maturity and are unlikely to have sustained hormone-induced oxidative stress. In the mouse, a delay in the start of treatment results in a reduced or even no effect. Most human PCa prevention trials were conducted on men aged 50 or more. In addition, the agent dose in animals is 50-80% of the maximally tolerated dose, whereas in humans lower doses may be required for bioethical reasons. The excellent review of Pienta et al. (Prostate Cancer Model Working Group) offers a list of limitations of preclinical models that have hampered the translation of their findings to human clinical trials [8].

<table>
<thead>
<tr>
<th>Agent*</th>
<th>Trial No.</th>
<th>Type</th>
<th>Institution</th>
<th>Phase</th>
<th>Status</th>
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</tr>
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Table 2. Clinical Trials of Preventive/Therapeutic Regimens for Prostate Cancer

9. Conclusions

Genetically engineered mouse models of PCa have paved the way to many important discoveries and helped to define the molecular events of prostate tumorigenesis. However, no single model precisely recapitulates all the molecular or cellular features of the progression of PCa from the normal gland to metastatic, hormone-refractory carcinoma, especially since its early stages are not those of single-cell-type disease, but must be viewed as a complex system of epithelial cells that display dysregulated growth within both a microenvironment composed of many cells which support such growth, and the host macroenvironment with its unique genotype and immune system. Further research is needed to better define these...
interactions, many of which are potential therapeutic targets. Several in vivo models can be utilized to study specific components of tumor initiation and progression. Meaningful interpretation of their results, however, demands a full understanding of the properties and limits of each model, along with employment of the model most consonant with the subject to be studied. Preclinical models have been poorly predictive of results in human studies because of both their inadequacy and their inappropriate use leading to the designing of clinical trials that do not mirror the preclinical model testing [106]. However, the chemoprevention field is particularly challenging since discrepancies have also been found between initial findings in several trials, secondary analyses and epidemiologic data, and subsequent randomized studies in humans [107]. These inconsistencies may reasonably be supposed to stem from the fact that dietary agents may act long before the scheduled commencement of a chemoprevention trial. Since such trials need to find outcomes (cancers), they invariably start with populations at higher risk of developing clinically detectable cancer, namely middle-aged and older subjects. However, dietary elements may either have a lifelong effect in their changes to the baseline risk for cancer or act at key points by priming the pump for its future development. In either case, dietary chemoprevention might be possible, but its indisputable demonstration in a trial would be highly unlikely. Do these discrepancies mean that all the preclinical and epidemiologic studies are wrong? It must primarily be considered that the timing of such interventions is unclear. Their employment in very high risk subjects, indeed, may actually be too late to significantly prevent cancer formation. Future studies will require both the use of other models founded on our increased understanding of human cancer proteomic genetics and epigenetics to define the very first steps in the progression of the disease and the ability of agents to impair or retard it, and a better “translational approach” achieved through preclinical studies that utilize the appropriate agent doses, and pharmacokinetic and pharmacodynamic parameters to take into account the differences in metabolism between mice and humans, together with clinical trials whose design takes account of how the preclinical testing was accomplished.

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Author details

Rossano Lattanzio\(^1\), Alessia Lamolinara\(^2\), Mauro Piantelli\(^1\) and Manuela Iezzi\(^2\)

1 Departement of Biomedical Sciences, University “G. D’Annunzio” Chieti, Italy

2 Department of Medicine and Aging Sciences, University “G. D’Annunzio” Chieti, Italy
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