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# Patient-Derived Mouse Models of Cancer

## Patient-Derived Orthotopic Xenografts (PDOX)

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Editor

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*This book is dedicated to the memory*
*of A.R. Moossa, M.D., and Sun Lee, M.D.*

Jørgen Rygaard (1934–2016)
Father of Patient-Derived Mouse Models of Cancer
and Modern Cancer Research

# Preface

Patient-derived xenograft mouse models of cancer are an area of intense research. This field has had a renaissance over the past 10 years after an almost quarter century of being ignored or denigrated as an irrelevant model. The current book gives a perspective on the long history of patient mouse models of cancer since the first paper by Rygaard and Povlsen in 1969. The book provides an overview of the state of the art of the field and especially emphasizes the importance of the use of orthotopic mouse models of patient cancer as these models enable metastasis to occur, which is the essence of clinical cancer. Chapters on patient-derived orthotopic xenograft (PDOX) cover the major cancer types. Other chapters cover important aspects of the use of patient-derived mouse models for cancer research and novel, transformative treatment. The last chapter previews an exciting future where patient-derived models are used for individualized more precise therapy on a routine basis.

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# 1 In Memoriam: Jørgen Rygaard (1934–2016), Father of Patient-Derived Mouse Models of Cancer and Modern Cancer Research

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The athymic "nude" mouse is possibly the most important tool in cancer research. The nude mouse has enabled the studies of human cancer in the laboratory *in vivo*. Nude mice were first discovered in 1962 in the laboratory of Dr. N. R. Grist at Ruchill Hospital's Brownlee Virology laboratory in Glasgow. The nude (*nu*) gene behaves as an autosomal recessive. The homozygotes, *nu nu*, are hairless (nude). Other parts of the syndrome initially observed were sulfhydryl group deficiency and abnormal keratinization of hair follicles [1]. All major types of human cancer have been grown and characterized in nude mice.

The nude mouse was first found to be athymic by Pantelourus [2] working in Glasgow, Scotland. Jørgen Rygaard spoke with a colleague in Denmark, Dr. Kresten Work, who had seen the nude mouse in an institute in Glasgow [3]. Rygaard asked Dr. Work what is the nude mouse used for? Work replied, "Nothing….they just keep them in a cage under the lab sink" [3]. Pantelourus observed that the nude mouse was athymic. Pantelourus also observed that blood leucocytes were low in the nude mouse which meant that the nude mouse was T-cell deficient, which explains why foreign tissue is not rejected by nude mice.

Nude mice are unable to mount many types of immune responses, including antibody formation that requires CD4+ helper T cells; cell-mediated immune responses, which require CD4+ and/or CD8+ T cells; delayed-type hypersensitivity responses (require CD4+ T cells); killing of virus-infected or malignant T cells (requires CD8+ cytotoxic T cells); and graft rejection (requires both CD4+ and CD8+ T cells) [1].

Nude mouse females have underdeveloped mammary glands and are unable to effectively nurse their young; therefore, nude males are bred with heterozygous (nu/+) females. In controlled, germ-free environments using antibiotic treatment, nude mice can live almost as long as a normal mouse (18 months to 2 years) [1].

Rygaard was able to arrange a shipment of nude mice from Scotland to Copenhagen which were carried in the cockpit of the British Airways plane from Glasgow. Rygaard then bred the nude males with normal NMRI mice from Bomholtgaard and with, brother-sister mating, was producing 50–100 nude mice per week at the SPF animal facility at the Copenhagen Municipal Hospital.

Having established the nude mouse colony, Rygaard asked his colleague Carl Povlsen (1940–1986) to obtain a tumor specimen from a colon-cancer surgery. Povlsen obtained a just-excised adenocarcinoma of the colon from a 74-year-old female. Small pieces from the sterile serosal side of the specimen were implanted subcutaneously into the flank of a nude mouse and the tumor grew. Even though the original donor patient had large metastasis in the liver, the tumor grew encapsulated (noninvasively) in the nude mice, an observation that numerous researchers would make on other subcutaneously-transplanted tumors in nude mice. Only when orthotopic (literally "correct place") models were developed were tumors able to metastasize in nude mice [3]. The nude mouse-grown tumors maintained the histology of the original patient's tumors, passage after passage. This is one of the greatest discoveries of cancer research—a patient's tumor could be grown and replicated indefinitely in a mouse. This discovery made human cancer research a feasible experimental science for the first time.

Rygaard donated breeding colonies of nude mice to NCI in Frederick, MD, to CIEA in Japan and to the Basel Institute for Immunology [3]. The nude mouse changed the paradigm of cancer research. Human tumors and human cancer cell lines could be grown systemically in an animal model for the first time.

Subcutaneous implantation readily allows observation of tumor take and growth. Rygaard's and Povlsen's patient tumor grew in all inoculated animals and reached a considerable size in the longest surviving animals. The mode of growth of the first s.c. human tumor xenograft was characteristic of what was observed later with other human patient tumors [3, 4]. The tumor was a local nodule and was encapsulated in a thin connective tissue capsule. The tumor was found to be mobile and free of the underlying fascia and covered with a network of vessels, both medium-sized and small arteries and veins. Upon histological examination, the tumor appeared to be similar to the patient's tumor. It was a well-differentiated adenocarcinoma [3]. Tumor tissue from this first implanted tumor was serially transferred to other nude mice, again inoculated s.c. and developed in the same manner. The tumor was maintained over 7 years for 76 passages [3, 5].

In 1972, Giovanella et al. [6] successfully transplanted a human melanoma cell line into a nude mouse. Numerous human cancer cell lines have been subsequently transplanted to nude mice. A large group of human patient cancers was transplanted directly from biopsy material into nude mice by Giovanella and his team [6].

Fiebig et al. have developed a very large bank of human patient tumors transplanted directly in nude mice. Initially, Fiebig et al. transplanted 83 human colorectal and 44 stomach cancers subcutaneously in nude mice. Tumor take was observed in 78 and 68%, respectively. Progressive tumor growth was found in 49 and 32%, respectively. Serial passage was performed in 46 colorectal, 17 stomach cancers, and four esophageal cancers. Tumor stage was the most important factor for the take rate. Metastatic tumors of the colon and stomach were grown in nude mice in 89% and 54%, respectively, which was significantly higher than in non-metastatic tumors. The take rate was independent of the degree of differentiation, the amount of fibrous tissue, sex, and tumor localization. The similarity of the xenografts in serial passage in comparison to the donor tumor was shown by histological and immunological examinations. Most of the xenografts were growing more rapidly in the serial passage than in early passages. Drug treatment of the human tumors in nude mice highly correlated with clinical response for the donor patients. Predictions for resistance (100%) and sensitivity (86%) validated the nude mouse for growth of human tumors and drug sensitivity testing (see Chap. 3) [7].

The majority of human tumors were implanted in nude mice in the subcutaneous space, a site which in most cases does not correspond to the anatomical tumor localization in the patient. Discrepancies between the invading and metastasizing abilities of tumors in their natural hosts compared to those of corresponding s.c. xenografts were repeatedly described [8].

The vast majority of human solid tumors, growing as subcutaneous grafts in the nude mouse, exhibited no metastasis which is generally associated with local expansive tumor growth and the presence of circumscribed tumor borders.

Wang and Sordat et al. [9] were among the first to determine whether the growth–regulatory properties of the tissue or organ site might induce changes in the expression of the invasive phenotype. Two human cancer cell lines of colonic origin, a moderately (Co112) and a poorly differentiated (Co115) carcinoma, were implanted as cell suspensions, both subcutaneously and within the descending part of the large bowel of *nu/nu* mice. In contrast to the well-circumscribed, pseudo-encapsulated subcutaneous tumors, Co112 and Co115 displayed a multifocal, micro- and macroinvasive growth pattern when implanted into the colon. Metastases were observed with the Co115 tumor. These were found in mesenteric lymph nodes and could be detected macroscopically. Vascular invasion by colon cancer cells was a constant finding and could be seen both for lymphatics and blood vessels. All these features, including the presence of some alterations of the microvasculature such as dilated thin-walled vessels described in human colorectal tumors, made the histopathology of these xenografts quite similar to the one reported for the original patient tumors. This seminal study indicated that tumor implantation at the orthotopic site, or site corresponding to the origin of the tumor in the patient, allows the tumor to behave in a similar manner as it did in the patient (see Chap. 4).

Subsequent studies from Fidler's laboratory and from others have shown that the implantation of human tumors in the orthotopic sites of nude mice can provide a suitable model of metastasis of human tumors [10].

Our laboratory has developed the technique of surgical orthotopic implantation (SOI) to transplant histologically intact fragments of human cancer, including tumors taken directly from the patient, to the corresponding (orthotopic) organ of immunodeficient rodents. SOI allows the growth and metastatic potential of the transplanted tumors to be expressed and reflects clinical cancer to a greater extent that when a suspension of cancer cells is implanted orthotopically [4, 8].

## Patient-Derived Orthotopic Xenografts (PDOX)

Discrepancies have been repeatedly described between the invading and metastasizing abilities of tumors in the patient compared to the benign tumor behavior in the subcutaneous-transplanted xenografts in nude mice as noted above. Human patient tumors rarely metastasize when grown subcutaneously in immunocompromised mice; this includes patient-derived xenograft (PDX) models. However, orthotopic implantation of intact tumor tissue can lead to metastasis that mimics that seen in patients. The patient-derived orthotopic xenograft (PDOX) models better recapitulate human tumors than PDX models. The PDOX nude mouse model was developed with the technique of SOI of intact cancer tissue. A greater extent of metastasis was observed in orthotopic models with implantation of intact tumor tissue compared with orthotopically implanted cell suspensions (e.g., in stomach cancer). This perhaps is due to the intact histology and cancer cell stroma interaction of the orthotopically implanted tumor tissue. PDOX models from patients with colon, pancreatic, breast, ovarian, lung, and stomach cancer and mesothelioma were established in the

early 1990s, resulting in primary and metastatic tumor growth very similar to that of the patient. PDOXS of model cervical cancer and sarcoma were recently developed, and metastasis in the PDOX models in reflects the metastatic pattern in the donor patient (see Chap. 7) [8].

## Transgenic Nude Mice Expressing Fluorescent Proteins in Almost All Tissues

We have developed the transgenic green fluorescent protein (GFP) nude mouse with ubiquitous GFP expression [11]. The GFP nude mouse was obtained by crossing non-transgenic nude mice with the transgenic C57/B6 mouse in which the β-actin promoter drives GFP expression in essentially all tissues. A nude mouse expressing red fluorescent protein (RFP) was also developed by our laboratory [12]. The RFP nude mouse was obtained by crossing non-transgenic nude mice with the transgenic C57/B6 mouse in which the β-actin promoter drives RFP (DsRed2) expression in essentially all tissues. The cyan (blue) fluorescent protein (CFP) nude mouse was also developed by our laboratory by crossing non-transgenic nude mice with the transgenic CK/ECFP mouse in which the β-actin promoter drives expression of CFP in almost all tissues (see Chap. 14) [13].

A PDOX pancreatic cancer was passaged orthotopically into transgenic nude mice ubiquitously expressing GFP and subsequently to nude mice ubiquitously expressing RFP. The tumors, with very bright GFP and RFP stroma, were then orthotopically passaged to non-transgenic nude mice. It was possible to image the brightly fluorescent tumors noninvasively longitudinally as they progressed in the non-transgenic nude mice due to the maintenance of the bright stroma throughout passages [14].

The GFP, RFP, and CFP nude mouse models provide unique understanding of the critical interplay between the cancer cells and their microenvironment within tumors especially when implanted with cancer cells expressing a different color fluorescent protein than the mouse.

Rygaard and colleagues also created the first “humanized” mouse. Various fetal tissues were transplanted to the nude mice by Rygaard and his colleagues. These fetal tissues were able to grow including the thymus, lung, pancreas, adrenal glands, kidney, testis, and ovary. The fetal tissues were transplanted subcutaneously [15, 16]. Perhaps if the fetal tissues were transplanted orthotopically, more types would have grown (see Chap. 20).

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# The Revival of Patient-Derived Xenograft Mouse Models of Cancer: Way Back to the Future

2

Robert M. Hoffman

Rygaard and Povlsen established the first patient tumor cancer nude-mouse model with a colon cancer surgical specimen. This was the first use of nude mice to grow a human tumor. During the 1970s and 1980s, there was much worldwide use of nude mice to grow both patient tumors and human cells lines. However, xenograft mouse models went out of fashion for almost 20 years after the introduction of "OncoMouse" in 1984, the first of a long line of transgenic mouse models of cancer. Halfway through the first decade of the present century, there was a revival of xenograft models that were basically the same as Rygaard and Povlsen's subcutaneous tumor model of 1969, in which the majority of human solid tumors do not metastasize. Orthotopic implantation of tumors enabled metastasis to occur. Although orthotopic metastatic mouse tumor models were first described in 1982 and further developed to be able to mimic metastasis in the patient in 1991, the use of orthotopic model remains limited despite their far superiority to subcutaneous or genetically-engineered mouse models of cancer in current and previous use.

Before the use of the athymic *nu/nu* mouse (nude mouse) for the growth of human tumors in 1969, there was no systematic way to grow human patient tumors in mice. Rygaard and Povlsen [1] implanted tumors in mice from a colon cancer from a 74-year-old patient subcutaneously (s.c.) in nude mice, which grew similar to the donor patient. The tumors grew locally and did not metastasize over 70 passages [1, 2].

Throughout the 1970s and 1980s, many authors noted that despite the metastatic behavior of tumors in the patient, s.c.-transplanted xenografts in nude mice were benign. This is still the case of PDX models today [2–4].

Wang et al. [5] in 1982 transplanted a human colon cancer cell line suspension orthotopically (literally "correct surface") in nude mice rather than "heterotopically"

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(literally "different surface," such as s.c.). A suspension of colon cancer cells was injected within the descending part of the large bowel of nude mice, resulting in metastases as well as local tumor growth [2]. However, orthotopic implantation of cancer-cell-line suspensions usually resulted in a low frequency of metastasis [2].

Patient-derived orthotopic xenograft (PDOX) models, which were implanted using intact tumor tissue with the technique of surgical orthotopic implantation (SOI) [2, 6], were established from patients with colon [7–9], pancreatic [10–21], breast [22], ovarian [23], lung [24], and stomach cancer [25] and mesothelioma [26] in the early 1990s in our laboratory, resulting in primary and metastatic tumor growth very similar to that of the patient [25]. Recently, PDOX models of sarcoma [27–31] have been developed, cervical cancer [32–34] as well as melanoma [35, 36].

A clinical study of 20 patients having stomach cancers that grew orthotopically in nude mice after SOI showed direct correlation of the metastatic pattern of the patient and mice [25]. In another case, a patient-derived colon cancer lung metastasis grew in the lung, but not in the colon or skin of nude mice [37].

In the 1980s, the Leder group published their famous "OncoMouse" paper [38] describing a transgenic mouse in which the normal mouse *Myc* gene was driven by a hormonally inducible mouse mammary tumor virus promoter to generate spontaneous mammary adenocarcinomas [38]. OncoMouse started the era of transgenic mouse cancer models, which would dominate the cancer mouse model field for almost 25 years. The tumors in these models were usually driven by "oncogenes" constructed with super-active viral promoters. More sophisticated techniques were later developed to establish transgenic tumor mouse models, including homologous recombination and the use of a Cre–*loxP* system for activating "oncogenes" or deactivating (knocking out) "tumor suppressor" genes [2].

The transgenic mouse models of cancer were touted as the "real" mouse models of cancer, and at the same time xenograft models were roundly denigrated and "xenograft" became a taboo word. For example:

Sharpless and Depinho [39] described these two reciprocal phenomena:

Their paper starts out by blaming the lack of effective drugs for cancer on xenograft models:

> "Most hold the view that the use of xenograft models in the cancer drug discovery and development process has proved to be problematic, with few predictive achievements and many notable failures."

Sharpless and Depinho [39] describe the problem:

> "Critics who comment on the failure of 'mouse models' are being dismissive of xenograft testing in particular" [39].
>
> "This approach [xenograft] has notable flaws, but because of its ease and low cost it has been used extensively in academia and the pharmaceutical industry during the past three decades…" [39]
>
> "The problem with xenograft analyses, however, is that many agents that show consistent and potent anticancer activity in specific xenograft models prove to be of limited use in the therapy of human cancer. This single fact is a major contributor to the low success rate of novel therapeutics when first tested in humans" [39].
>
> "Third, and perhaps most significant, is the fact that these systems [xenograft] model cancer as if it was [sic] a disease of homogeneous rogue cells" [39].

> "By failing to recapitulate the complex and evolving tumor-host stroma interactions, which could be further complicated by the immunodeficient state of the animal, xenograft analyses are reductionist and fall short of fully capturing the potent modulating effects of the tumor microenvironment in drug response" [39].
>
> "…xenograft studies typically use only a few human tumor cell lines, the oncogenomic profiles of which represent only isolated combinations of the wide spectrum of genetic and epigenetic mutations that are resident in a given tumor type presented in the clinic" [39].
>
> "As the specific genetic profile can alter a tumor's response to a drug… the inability to predict the outcome of clinical trials probably results in part from a failure to represent the enormous genetic diversity of tumors in patients [by xenografts]" [39].
>
> "…novel inhibitors of angiogenesis (endostatin and angiostatin) showed potent anticancer activity when given alone or in combination against a large variety of xenografted human and murine cell lines, but so far have not demonstrated single-agent activity in human cancers…" [39]
>
> "…by the observation that most compounds entering human clinical testing fail because of lack of efficacy, despite showing promise in preclinical xenograft testing" [39].

These "criticisms" of xenograft models by Sharpless and Depinho were typical of what was said in published scientific papers and in scientific meetings by leaders of the transgenic mouse field. In major meetings on mouse models of cancer, xenograft presentations were discouraged or not allowed. The National Cancer Institute's "Mouse Models of Human Cancer Program" was funding essentially only grant applications on transgenic mouse models of cancer. Thus, for approximately a quarter century, the great work of mouse xenograft models of cancer, especially human patient tumor xenografts (see later chapters in this book), was ignored or described as worthless and blamed for the failure to find effective drugs for cancer.

In 2006, a "way back to the future" event occurred, all the way back to 1969, Rygaard and Povlsen [1]. The s.c.-transplanted human patient xenograft mouse model was heavily promoted by Hidalgo et al. [40] and his company, Champion's. At first, the term "tumorgraft" [41] was used so as not to use the taboo term "xenograft" [2].

The reborn s.c. models sometimes used more immunodeficient, such as nonobese diabetic, severe combined immunodeficiency (NOD-SCID) mice. However, the tumors were still s.c. and did not metastasize. In order not to seem to be going back to the 1960s, the born-again s.c.-transplanted mouse models were given even more exotic new names such as "xenopatients" or "avatars" [4] in order to exaggerate their capability and novelty [2]. The October 3, 2014 issue of *Science* had an "avatar" on the cover, which stated: "To make mice better mirrors of human cancer, researchers are building 'avatars' with the cancer of a particular patient…. The work marks a sea change in cancer biology and is stirring hope that new mouse models will pave the way to more personalized care" [42]. However, orthotopic patient models are hardly mentioned in the "xenopatient" and "avatar" papers [2, 4, 43]. Patient-derived xenografts, simply referred to as PDX models of cancer, are now the hot fad and "transgenic" cancer models appear to be in eclipse [2].

After 52 years in science, I have seen many scientific fads that come and go and come back again [2, 44]. We are now back to the late 1960s with the so-called patient-derived xenograft "PDX" model. Orthotopic models attained a modicum of popularity in the late 1980s and early 1990s [2], due in large part to the great efforts of Fidler [45]. It seems that most cancer researchers have either forgotten about or

are unaware of orthotopic models [2], especially PDOX models, which are metastatic and resemble the patient's tumors [2]. It is the goal of the present book to give a better appreciation of the history as well as state of the art of patient mouse models of cancer, in particular the patient-derived orthotopic xenograft (PDOX) models.

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# Patient-Derived Xenograft Models for Human Cancer: The Freiburg Experience

3

Heinz-Herbert Fiebig

Since 1969, human patient patient-derived xenograft (PDX) have been grafted into immune-compromised mice, and until today they are the most important model system to evaluate novel compounds against cancer and to study tumor biology. In Freiburg more than 3.000 patient tumors have been transplanted subcutaneously into nude mice from which 450 PDX have been established and selected as permanent tumor models. In 90% of them the molecular profile was determined including gene expression, mutations by WES and copy number variations. 250 models were characterized for their sensitivity against targeted and cytotoxic drugs in-vivo and also in-vitro in 3D cultures. Based on the testing results predictive gene signatures and biomarkers were investigated for small molecules and antibodies. A comprehensive data base of all molecular and sensitivity data allows the selection of suitable tumor models for investigating new drugs.

## Historical Aspects of PDX

Since 1969, human PDX models have been developed in immune-suppressed mice, and until today they are the most important model system to evaluate novel compounds against cancer and to study tumor biology. The growth of human tumors in a murine host was only possible when a specific mouse mutant was discovered in Glasgow more than 50 years ago.

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## Origin and Properties of Nude Mouse

Early in the 1960s, Isaacson and Cattanach [1] observed in an albino mouse strain a spontaneous mutant which was hairless and had a very short life span. Flanagan described it in 1966 [2]. Because of the hairlessness, he called the mutant "nude" and introduced the symbol "nu." The mutation was autosomal recessive. Flanagan correlated the nu mutation with known chromosomal markers and found that the mutation nu was located at the chromosomal group VII between the markers Re and Tr. Later on the genes Re-nu-Tr were localized on chromosome 11 of the mouse [3].

The hairlessness was always connected with an aplasia of the thymus [4]. With this discovery, the nude mouse proved to be an excellent model to study the thymus function. The growth of allo- and xenogeneic transplants allowed novel applications in research.

Rygaard and Povlsen reported the first successful transplantation of a human tumor in the nude mouse, namely, an adenocarcinoma of the colon [5]. Other groups transplanted various human tumor types which could also be transferred in serial passage [6, 7]. Also human tumor cell lines being established in tissue culture grew successfully in nude mice; they formed solid tumors after subcutaneous transplantation [8, 9]. The treatment of a Burkitt lymphoma and a melanoma growing in serial passage were reported by the Copenhagen group [10, 11].

Rygaard performed the first xenogeneic transplantation of skin from Wistar rats into nude mice [12]. Further successful transplantations were described with the skin of hamsters and rabbits [13], cats [14], birds [15], and also human skin [16, 17]. The skin of snakes and frogs was not rejected. They showed degenerative changes which were explained by the unphysiological environment [14, 15].

The immunological properties of the nude mouse were further characterized by several research groups. Tumor biologic and therapeutic investigations were also carried out. The findings were presented and summarized at international symposia starting in 1973 in Aarhus, Denmark; 1976 in Tokyo; 1977 in Columbus, USA; and 1979 in London, in Frankfurt, and in Bozeman, Montana, USA [18–22].

The athymic mouse has also opened new possibilities for microbiologic and parasitological studies. Models of lepra and *Pneumocystis carinii* were developed [23–25], and investigations on immune reactions during infections were of special importance. Humoral and cellular mechanisms were studied after infections with *Plasmodium berghei* [26, 27], *Trypanosoma musculi* [28], helminths [29–31], and different virus infections [32].

## Morphologic and Physiologic Characteristics

The nude mutation has a number of consequences of which the aplasia of the thymus is the most important. The initially described complete aplasia of the thymus was not confirmed. Pantelouris and Hair published in 1970 the existence of a rudiment of the thymus, which was confirmed by other groups [33–36]. In the anterior mediastinum, two small lobs with residual components of the thymus were detected [37, 38], and the thymus is much smaller compared to immunocompetent mice [39].

Homo- and heterozygous nude mice have an impaired hematopoiesis. Zipore and Trainin [40] and Holub et al. [41] found that the hematopoietic stem cells have a reduced capability to form colonies. Since also heterozygous mice are defective, Dolenska et al. [42] concluded that these defects are present on stem cells and due to a mesenchymal defect of the mutation and not secondary to the defect of the thymus. Nude mice showed in the peripheral blood a leukopenia of 25–30% of the normal value. In heterozygous mice a reduction to the half of the normal value was already reported by Pantelouris [4]. The leukopenia is caused by lack of mature T lymphocytes [43, 44].

The most obvious effect of the mutation nu is the hairlessness. The nude mouse has functional hair follicles, but the keratinization is impaired resulting in braking up of the hair [2]. The hairlessness results in a number of physiological properties. Nude mice have a lower body temperature, a higher metabolic turnover, and lower blood sugar levels compared to heterozygous mice [45–47]. Nude mice have a higher loss of water through the skin and homozygous female drink 2/3 more than heterozygous haired mice [17]. The other organ systems of the nude mouse are developed in a normal way.

## Immunological Properties

Precursors of T lymphocytes are present in the nude mouse, but due to the thymus aplasia, they do not mature into functional T lymphocytes [48–50]. For instance, the lymph of the ductus thoracicus of nu/nu mice contains only B lymphocytes, whereas 85% T lymphocytes and 15% B lymphocytes are found in immunocompetent mice [51]. Nu/nu mice have an unusual high amount of natural killer cells compared to heterozygous mice or mice without mutation. They are mainly found in the spleen. Natural killer cells seem to play an essential role in the residual immune response of the nude mice, e.g., against the rejection of leukemias and lymphomas. An even higher amount of natural killer cells seen after opportunistic infections can result in a lower take rate and growth of transplanted tumors. Macrophages of the nude mouse have also cytotoxic properties which may play a role in the rejection of tumors [52].

The B-cell population of lymphocytes shows a normal development. The total immunoglobulin concentration is similar to immunocompetent mice. The fractions are slightly different: IgG and IgA are reduced in nude mice and IgM increased [46, 53, 54]. Functional tests showed that the humoral immune reaction is decreased which needs thymus-dependent T helper and T suppressor lymphocytes. A normalization of the humoral and cellular defense occurs after T-cell substitution or thymus transplantation [55, 56], and also large tumor masses were rejected [57].

Due to the missing T lymphocytes, nude mice have a high risk acquiring infection of bacteria or viruses which are not pathogens for immunocompetent strains. Infections have been reported mainly mouse hepatitis virus and noroviruses as well as *Staphylococcus aureus* resulting in skin and eye abscesses.