Assessment of Chimerism in Epithelial Cancers in Transplanted Patients

Christophe Leboeuf^{a, b} Philippe Ratajczak^{a, b} Jérôme Vérine^{a–c}
Morad Elbouchtaoui^{a, b} François Plassa^{a, b, d} Luc Legrès^{a, b}
Irmine Ferreira^{a, b} Wissam Sandid^{a, b} Mariana Varna^{a, b}
Guilhem Bousquet^{a, b} Laurence Verneuil^{a, e} Anne Janin^{a–c}

^aInserm, U728-Paris, ^bLaboratoire de Pathologie, UMR_S728, Université Paris Diderot, Sorbonne Paris Cité and Departments of ^cPathology and ^dBiochemistry, AP-HP, Hôpital Saint-Louis, Paris, and ^eDermatology, CHU-Caen, Caen, France

Key Words

Chimerism · Allogeneic transplantation · Epithelium · Squamous cell carcinoma · Adenocarcinoma

Abstract

Cancer is now the most severe complication in the long term in transplant recipients. As most solid-organ or hematopoietic stem-cell transplantations are allogeneic, chimerism studies can be performed on cancers occurring in recipients. We summarize here the different methods used to study chimerism in cancers developing in allogeneic-transplant recipients, analyze their respective advantages and report the main results obtained from these studies. Chimerism analyses of cancers in transplant recipients require methods suited to tissue samples. In the case of gender-mismatched transplantation, the XY chromosomes can be explored using fluorescent in situ hybridization on whole-tissue sections or Y-sequence-specific PCR after the laser microdissection of tumor cells. For cancers occurring after gender-matched transplantation, laser microdissection of tumor cells enables studies of microsatellite markers and high-resolution melting analysis of mitochondrial DNA on genes with marked polymorphism, provided these are different in the donor

and the recipient. The results of different studies address the cancers that develop in both recipients and in transplants. The presence of chimeric cells in these two types of cancer implies an exchange of progenitor/stem-cells between transplant and recipient, and the plasticity of these progenitor/stem-cells contributes to epithelial cancers. The presence of chimeric cells in concomitant cancers and preneoplastic lesions implies that the oncogenesis of these cancers progresses through a multistep process.

© 2014 S. Karger AG, Basel

Introduction

Organ transplantations are now common [1–4] and they constitute a significant improvement in the matters of public health and the quality of life for patients. Progress in the optimization of donor/recipient immunocompatibility matching [5, 6] and immunosuppression protocols [7, 8] has led to a significant decrease in the number and severity of acute graft rejection episodes.

The most severe complications now occur in the chronic stage, with the emergence of cancers in the long term in transplant recipients [9]. The number of these

cancers is increasing along with the increase in the numbers of transplantations performed and patients with long-term transplantation [10]. The emergence of cancer can decrease the overall prognosis of the transplantation process and, therefore, the benefit of this type of therapy for the patient [11].

Most solid-organ or hematopoietic stem-cell transplantations are allogeneic [1–3], and chimerism studies are therefore appropriate [12, 13]. After hematopoietic stem-cell transplantation, tests for chimerism are used in the follow-up of the patient, and different methods for chimerism studies in blood and tissue have been validated. When applied to the cancers developing in transplant recipients, these methods can identify the donor/recipient origin of the different cellular components of the tumor [12, 14]. When these methods are applied to preneoplastic and neoplastic lesions, they offer clues to the complex process of the oncogenesis of these tumors.

Different Types of Cancer in Transplant Patients

Cancers Developing in Transplant Recipients

Kidney and liver transplantations are the most commonly performed solid-organ transplantations. In addition, kidney transplantation is the longest-standing [15], so the number of patients with long-term kidney transplantation is large [16]. In the period 2005–2008, the United Network for Organ Sharing in the USA totaled 32,258 kidney transplants, and the Collaborative Transplant Study in Europe totaled 23,530 [16].

In kidney transplant recipients, the most common cancer occurring after transplantation is nonmelanoma skin carcinoma [17]. These tumor types are also the most frequent after liver and heart transplantation [18]. Recent studies have classified the cancers occurring in transplanted patients according to their infectious or noninfectious origin. According to this classification, of the infection-related cancers, liver malignancies, Kaposi sarcoma and EBV-related non-Hodgkin lymphoma have the highest incidence when all solid-organ transplantations are considered [19]. Among noninfection-related malignancies, lung and kidney cancer, melanoma and nonmelanoma skin cancer have the highest incidence when all solid-organ transplantations are considered [19]. The highest incidence of lung cancers occurs in lung transplant recipients, possibly because of smoking, but an increased risk of lung cancer is also associated with HIV infection, independent of the patient's smoking history [20, 21]. Numerous similarities have been found in the

incidence of cancer in HIV-AIDS patients compared to immunosuppressed transplant recipients, particularly for cancer with a known infectious cause, as in human papillomavirus-related cancer and liver cancer [22, 23].

Cancers that Develop in the Transplants

Cancers developing in the grafts have been reported in kidney, liver and lung transplants.

De novo tumors in renal allografts are rare and their prevalence is estimated at between 0.19 (calculated on a series of 41,806 kidney transplant recipients [24]) and 0.46% (calculated in a series of primary renal cell carcinomas occurring after kidney transplant [25]). One constant characteristic of primary cancers developing in transplanted kidneys is the predominance of the papillary renal-cell carcinoma type, whereas the clear-cell renal-cell carcinoma type is the most frequent renal cancer in the general population. A multicenter French study on cancers in kidney transplants showed an incidence of 45.6% for papillary renal cell carcinomas (vs. 10–15% in the general population), most of them being confined to an organ (T1–T2) and of a low grade (G1–G2) [24]. In a German retrospective examination of 2,001 consecutive renal transplant recipients, 1.5% renal cell carcinomas were found; 25 tumors were found in native kidneys and 5 in allografted kidneys. The rate of papillary renal cell carcinoma was 37.5% [26]. Another characteristic of the primary cancers occurring in kidney transplants is the concomitant occurrence of other lesions in the kidney allograft, e.g. angiomyolipoma [27], or often papillary adenoma proliferations [28]. Clinically, the papillary renal cell carcinomas in kidney transplants occur in patients who were younger at the start of immunosuppression, and they are aggressive despite their low-stage and low-grade classification [26]. When a tumor of the renal-cell carcinoma type occurs in a kidney transplant, it is important to demonstrate that it is not a metastasis from a renal cell carcinoma developing in the native kidneys. This can be achieved with clinical data and imaging studies [29]. In imaging diagnosis of these cancers, ultrasonography sensitivity is reduced by cystic transformation of the native kidneys after renal failure [26].

Following liver allograft transplantation, the main cause of the development of hepatocellular carcinoma (HCC) in the transplant is actually HCC recurrence, a problem that occurs in approximately 20% of patients after transplantation, despite refined selection criteria and exhaustive preoperative staging [30]. However, de novo HCC in liver grafts have also been observed in transplanted patients with no previous evidence of HCC [31–33].

Table 1. Different methods used for chimerism study in cancer tissue

Molecular target	Technique	Reference	Quantitative	Requires microdissection of tumor cells	Limitations
X/Y chromosomes	FISH	[12, 27, 83] [14, 84]	Yes	No	only after gender-mismatched transplantation
	ISH	[85] [54, 73] [86, 87]	Yes	No	
Y-sequence-specific PCR amplification	PCR	[88]	Semi-quantitative	Yes	exclusion of female patients who have been pregnant with a male baby
Microsatellite marker	FISH	[89]	Yes	No	only if donor and recipient DNA are available choice of microsatellite in highly polymorphic genes different for donor and recipient
	PCR	[14, 33, 83] [54, 73]	No	Yes	
D-loop of mitochondrial DNA	DNA HRM	[53, 54, 73]	No	Yes	only if donor and recipient DNA are available

These true de novo HCC can be associated with recurrent hepatitis B [34] or with sustained hepatitis C virus clearance after liver transplantation [35]. They can also be associated with cirrhosis linked to recurrent hepatitis B [36] or C. To distinguish de novo HCC from recurrent HCC, a useful clinical element is the mean time-lapse to occurrence, which is around 5 years after liver transplantation for de novo HCC as opposed to approximately 2 years for recurrent HCC [35].

Cancers developing in lung transplants are squamous cell carcinomas, adenocarcinomas or more rarely bronchoalveolar carcinomas and sarcomas. In 2 separate studies, the incidence of these bronchogenic cancers in lung transplants was 2.4% in 290 transplant recipients [37] and 2.6% in 345 transplant recipients [38]. The average interval from transplantation to the development of lung cancer is 5 years. These cancers occurred in patients with a history of smoking [37, 38] and are usually diagnosed at an advanced stage and have a poor outcome [37].

Cancers Transferred with the Transplants

Cancers of donor origin in transplant recipients can be transferred with the transplant. This is a rare event. Its occurrence is evaluated at between 0.05 [39] and 0.02% [40].

Transfers of malignant melanoma and choriocarcinoma, two tumors with a high hematogen metastatic potential, have been reported with kidney transplants [41, 42]. In the UK Registry [39], renal cell carcinomas have also been found to be transmitted in kidney transplant recipients, non-small cell lung cancer in lung transplant recip-

ients and colon adenocarcinoma and neuroendocrine tumors in liver transplant recipients. One major challenge for solid-organ transplantation teams is the approval of patients with primary brain tumor to be donors, because the extraneural spread of primary brain tumors is rare and there is a critical shortage of donors for transplantation. One study found that glioma cells were transferred in this manner in a pancreas transplant [40].

After allogeneic bone marrow transplantation, malignant diseases transferred with the transplant include leukemia [43], Hodgkin lymphoma and non-Hodgkin lymphoma [44, 45].

Different Methods for the in situ Chimerism Study of Cancers in Transplant Patients

Table 1 shows the different methods used for chimerism studies, which are currently used in the follow-up of patients after hematopoietic stem-cell transplantation. These studies are performed on blood cells using the microsatellite markers of highly polymorphic genes. The first step is the analysis of the donor and recipient DNAs before the transplantation, using a series of microsatellite markers with short tandem repeat sequences of different lengths. Microsatellite markers are chosen only when donor and recipient DNAs before the transplantation are different.

As a second step, the selected microsatellite markers are tested at different times after transplantation in order

to monitor the number of chimeric cells (in most instances, recipient-derived) in the blood of the recipient. Decreasing values of donor chimerism are predictive of transplant failure and relapse of disease [46].

The reported average sensitivity of this method in detecting minor cell populations is not >5%, but it is highly specific and can easily be quantified in a given number of blood cells [47].

These methods using microsatellite markers can be transposed for chimerism studies of solid cancers, using *in situ* hybridization or PCR after the laser microdissection of tumor cells.

Fluorescent *in situ* hybridization (FISH) for microsatellite markers can be performed on fixed- and frozen-tissue sections. Not all fluorescent probes for microsatellite markers of highly polymorphic genes are commercially available and they may have to be designed and labelled [48]. On tissue sections studied with FISH, the number of positive cells per field is easy to quantify. The type of positive cells in the tissue sections can also be identified either from morphologic criteria or by using immunostaining combined with FISH [14]. The transposition of PCR methods for blood cells to analyze cancer tissue requires the selection of tumor cells, which can be performed using laser microdissection [49]. However, a minimum number of tumor cells have to be microdissected to perform the PCR and its controls. Since laser microdissection is currently performed on 7- μ m-thick tissue sections, sections of 500–1,000 tumor cells are necessary [49]. In the case of tumors associated with an extensive inflammatory reaction, it is necessary to check the quality of the laser-microdissected cell population using PCR with an epithelial marker and a pan-leukocyte marker to be sure that no inflammatory cells have been microdissected along with the tumor cells. DNA extraction from these small quantities of biological material is optimized using specific kits [50]. The microsatellite probes and PCR protocols are similar to those used for blood cells [47]. Similarly, the method of high-resolution melting (HRM) of mitochondrial DNA, currently used for filiation inquiries [51] on cells in suspension, can be transposed to chimerism studies of cancer tissue sections [52]. For this type of analysis, the molecular target is the D-loop of the mitochondrial DNA. This method requires the availability of donor and recipient DNA and a selection of tumor cells using laser microdissection of tissue sections. As for microsatellite analysis, a minimum number of microdissected tumor cells are required for the PCR and its controls. The molecular probes and PCR protocols are similar to those performed on cell suspensions.

The new droplet digital PCR (ddPCR) can enable both the identification of chimeric cells and relative counts of chimeric cells in a given number in microdissected cells. In the context of gender-mismatch transplantation, ddPCR can identify Y-bearing cells in female recipients in the same way as classic PCR. ddPCR is an emulsion-based PCR process which performs absolute quantification of nucleic acids. The first step is the division of a duplex fluorescent-probe-based PCR assay into approximately 20,000 highly uniform micelles with a volume of 1 nl. Each droplet in the emulsion is an independent nano-PCR. After PCR, the droplets are focused into a single-file beam of droplets which flows through a cytometer under LED excitation [53]. This enables enumeration of PCR-positive and PCR-negative droplets. In the context of cancer in transplant patients, ddPCR can thus identify the number of Y-bearing cells in a given number of microdissected cells.

Results and Discussion

The main result obtained from chimerism studies of cancers in transplant recipients is the identification of the chimeric cells within these cancers. This identification enables discussion on (1) the migration of the chimeric cells into the cancer, and particularly on what the triggering factor initiating this migration could be and (2) which type of cell is at the origin of the chimeric cells. In kidney transplant recipients, donor-derived cells were first identified in a basal cell carcinoma occurring in sun-exposed areas of the skin [12]. The method used was the detection of the Y chromosome in female recipients of male kidney transplants. Conversely, recipient-derived cells have been identified in renal cell carcinomas that developed in a kidney transplant. In two different studies [29, 54], the method used was a comparison between recipient blood and renal-cell carcinoma microsatellites to confirm the recipient origin of neoplastic cells.

The identification of chimeric cells in cancers in transplant recipients, either in the recipient's skin or in the kidney transplant, demonstrates that a migration of stem-/progenitor cells has occurred. The triggering factor for chimeric cell migration in these cancers could be a process of inflammation and tissue repair. In nonneoplastic tissue, particularly in the microvessel network, chimeric cells have been found at repair sites. In the 3 months following kidney transplantation, donor-derived endothelial cells have been identified in the vessels of the kidney transplant, close to the surgical suture [55]. In the 100 days following bone marrow transplantation,

donor-derived cells were found in the microvessels and epidermis of patients, only if they had undergone acute graft-versus-host disease. In addition, the numbers of apoptotic epidermal or endothelial cells were significantly related to the numbers of chimeric cells in the epidermis or microvessels, thus confirming the relationship between the intensity of tissue damage and the density of chimeric cells. In solid-organ transplant recipients, no graft-versus-host disease occurs, but the transplantation process itself induces tissue damage. After human lung transplantation, chimeric cells in the pulmonary epithelium [56] as well as in the endothelium of microvessels [57] have been found to be associated with transplant rejection. The participation of recipient-derived cells in endothelial repair has also been reported in liver [58] and heart [59] transplants. Two mechanisms have been described in endothelial cell repair: the local migration and proliferation of endothelial cells adjacent to the site of injury and the homing and incorporation of endothelial progenitor cells at this site [60]. Unlike the endothelium, the epithelium can be pluristratified, but the homing and incorporation of progenitor cells at the site of injury is also observed when epithelial progenitor cells become exhausted by severe or chronic injury [61, 62].

Even if the migration of a progenitor cell is demonstrated by the identification of chimeric cells in cancers of transplant recipients, the type of progenitor cell involved has still to be established. In cancers occurring in bone marrow transplant recipients, hematopoietic stem cells are infused. These can be the origin of rare but authentic donor-derived posttransplant leukemia. Since the original description in 1971, more than 50 cases of donor-cell leukemias have been reported and considered to be the result of the oncogenic transformation of apparently normal donor hematopoietic cells in the transplant recipient [43]. The occurrence of epithelial carcinoma of donor origin after bone marrow transplantation implies a supplementary step of transdifferentiation of marrow stem cells in normal epithelial cells [13, 14, 63]. It has been shown that bone marrow cells contribute to epithelial cancers of the small bowel, colon, lung and skin [64]. The possibility that these cancers might stem from mesenchymal stem cells that are coinfused with hematopoietic stem cells within the marrow transplant cannot be excluded. In a kidney transplant recipient, we were able to demonstrate the presence of the same p53-mutation in the tumor cells of a skin squamous cell carcinoma and in epithelial tubular cells in the kidney transplant in a biopsy performed 7 years before the occurrence of the skin squamous cell carcinoma [65]. To date, this is the only observation dem-

onstrating the contribution of donor epithelium to the epithelial cancer in the recipient.

With regard to the cancers occurring within the transplants, in grafted kidneys, the majority are papillary renal cell carcinomas [24], i.e. epithelial tumors. The presence of recipient cells in these cancers, found in a series of human *de novo* papillary renal cell carcinomas [54], opens up discussion about the type of recipient cells that homed to the transplant cancer. The first hypothesis is homing to metastatic cells from a papillary renal cell carcinoma developing in one of the native kidneys when these have not been removed. Another possibility is the engraftment of exfoliated normal tubular cells from the native kidneys coming into the transplanted kidney via a mechanism of vesicoureteral reflux. In kidney transplant recipients, as in normal individuals, thousands of exfoliated renal tubular epithelial cells are shed into the urine each day [66]. These are able to establish viable cultures [67, 68] and form nephrogenic adenoma [69]. Finally, the migration and homing of circulating, normal recipient-derived progenitor cells to the transplanted kidney could occur. Populations of progenitor/stem cells resident outside the organ are able to contribute to the renewal of different lineages, even in the tissue from a separate germ layer [70].

Preneoplastic lesions can occur along with cancers in transplant recipients. The intent of the chimerism studies of preneoplastic lesions and cancers both occurring in the same patients is to assess whether there is a homing of progenitor cells at the early preneoplastic stage, and to compare the number of chimeric cells in the lesion, in the cancer and in the normal skin, all in the same patient.

In kidney transplant recipients, the main type of cancer is squamous cell carcinoma occurring on sun-exposed areas of the skin [9]. These squamous cell carcinomas are multiple and are often associated with multiple actinic keratosis [71, 72]. In 2 female recipients of male-kidney transplants who had noncontiguous actinic keratosis and squamous cell carcinoma and for whom tissue samples and DNA from both donors and recipients were still available, we conducted a chimerism study using four independent methods: FISH for X and Y chromosome detection, ZFYqPCR, polymorphic microsatellite analysis and mitochondrial DNA HRM analysis [73]. p16INK4A immunohistochemistry (clone JC8, Biocare-Medical) and *in situ* hybridization using an INFORM HPV III Family 16 Probe (Roche-Diagnostics, Basel, Switzerland) showed the absence of oncogenic HPV expression in these squamous cell carcinomas. XY FISH combined with cytokeratin on the same tissue section showed chimeric XY cells in the basal-layer actinic keratosis and in the basal layer and in-

vasive areas in squamous cell carcinoma. No chimeric cell was found in normal surrounding skin. Count showed a mean percentage of 4.5% of chimeric cells in the squamous cell carcinoma studied and 2% in the actinic keratosis studied. Laser microdissection, performed on tissue sections after immunostaining inflammatory cells, enabled us to collect only keratinocytes from the basal layer of the actinic keratosis and keratinocytes from the basal layer and invasive areas in the dermis of the squamous cell carcinoma. The polymorphic microsatellite marker analyses and mitochondrial DNA HRM performed on the laser-microdissected cells also showed the presence of cells of donor origin in the squamous cell carcinoma and actinic keratosis. The distribution of chimeric cells in the basal layer and invasive areas of the squamous cell carcinoma corresponded to the 'outer proliferating layer' where tumor-initiating cells were recently characterized in skin squamous cell carcinoma [74]. Chimeric cells were also found in the basal layer of actinic keratosis, a disease usually considered as benign, although molecular studies have shown a frequent loss of heterozygosity [75]. The presence of chimeric cells in actinic keratosis and squamous cell carcinoma, and not in the normal surrounding skin, could be linked to the remodeling of tissue accompanying disease progression, as progenitor cells are recruited to the sites of skin injuries in experimental conditions [76] and tissue repair mechanisms in common with stem-cell renewal in carcinogenesis [77]. The detection of chimeric cells at the stage of actinic keratosis is in favor of a multistep process in the progression from actinic keratosis to squamous cell carcinoma.

Regarding cancer in transplant recipients that has developed in the transplant, papillary renal cell carcinoma has a high incidence in kidney allografts [24]. It is frequently multifocal [78] and is associated with papillary adenoma, an epithelial lesion considered as benign and morphologically indistinguishable from papillary renal cell carcinoma, except by its size (<5 mm) [79].

As for actinic keratosis and squamous cell carcinoma occurring in the skin of kidney transplant recipients, the concomitant development of multiple tumors and the papillary adenoma-carcinoma association make papillary renal cell carcinoma an interesting model to gain insight into the evolutionary process of human cancer. Two models have been proposed: either cancer progenitor cells drive tumor initiation and progression (cancer stem-cell model) or over time, a premalignant or malignant cell population acquires multiple mutations, genetic instability and uncontrolled proliferation (clonal evolution model) [80–82].

In 5 kidney transplant recipients with de novo papillary renal cell carcinoma and papillary adenomas in kidney transplants, XY FISH, polymorphic microsatellite DNA and mitochondrial DNA HRM analyses on laser-microdissected tumor cells identified recipient-derived cells in both papillary cell carcinoma and papillary adenoma. Counts were performed on XY FISH sections. The proportion of tumor cells with recipient chimeric cell characteristics was 90.2–94.5% in papillary carcinomas and 94.1–98.8% in the papillary adenoma. We did not find tumor cells with donor characteristics in these recipient-derived papillary renal tumors [54]. These data demonstrated an identical origin for the precursor cells in the two lesions, which were located in the same original microenvironment and had the same cellular aspect, and thus had the same degree of cellular differentiation. There were differences in size, the benign tumors being smaller than the malignant ones, and in molecular characteristics, the malignant tumors having additional genetic alterations. All these elements are in favor of a multistep molecular process [80, 81] leading to papillary renal cell carcinoma.

In conclusion, chimerism assessment in epithelial cancers in transplanted patients identified a homing of progenitor cells of donor origin to recipient cancers and, conversely, a homing of progenitor cells of recipient origin to transplant cancers. It also showed a homing of these cells to associated premalignant lesions, a fact in favor of successive steps in the carcinogenesis of these epithelial cancers.

Acknowledgments

The authors would like to thank the Tumorothèque of Hopital Saint Louis, J.M. Cayuela for critical review of the manuscript and A. Swaine for reviewing the English version.

References

- 1 Colvin-Adams M, Smith JM, Heubner BM, Skeans MA, Edwards LB, Waller C, Snyder JJ, Israni AK, Kasiske BL: OPTN/SRTR 2011 annual data report: heart. *Am J Transplant* 2013; 13(suppl 1):119–148.
- 2 Matas AJ, Smith JM, Skeans MA, Lamb KE, Gustafson SK, Samana CJ, Stewart DE, Snyder JJ, Israni AK, Kasiske BL: OPTN/SRTR 2011 annual data report: kidney. *Am J Transplant* 2013;13(suppl 1):11–46.
- 3 Valapour M, Paulson K, Smith JM, Hertz MI, Skeans MA, Heubner BM, Edwards LB, Snyder JJ, Israni AK, Kasiske BL: OPTN/SRTR 2011 annual data report: lung. *Am J Transplant* 2013;13(suppl 1):149–177.

- 4 Transplantation TEGfBaM: EBMT Registry.
- 5 Brand A, Doxiadis IN, Roelen DL: On the role of HLA antibodies in hematopoietic stem cell transplantation. *Tissue Antigens* 2013;81:1–11.
- 6 Roelen DL, Doxiadis II, Claas FH: Detection and clinical relevance of donor specific HLA antibodies: a matter of debate. *Transpl Int* 2012;25:604–610.
- 7 Nordlander A, Uhlin M, Ringden O, Kumlien G, Hauzenberger D, Mattsson J: Immune modulation to prevent antibody-mediated rejection after allogeneic hematopoietic stem cell transplantation. *Transplant Immunol* 2011;25:153–158.
- 8 Zdanowicz MM: The pharmacology of immunosuppression. *Am J Pharm Educ* 2009;73:144.
- 9 Euvrard S, Kanitakis J, Claudy A: Skin cancers after organ transplantation. *New Engl J Med* 2003;348:1681–1691.
- 10 Mudigonda T, Levender MM, O'Neill JL, West CE, Pearce DJ, Feldman SR: Incidence, risk factors, and preventative management of skin cancers in organ transplant recipients: a review of single- and multicenter retrospective studies from 2006 to 2010. *Dermatol Surg* 2013;39:345–364.
- 11 Ison MG, Nalesnik MA: An update on donor-derived disease transmission in organ transplantation. *Am J Transplant* 2011;11:1123–1130.
- 12 Aractingi S, Kanitakis J, Euvrard S, Le Danff C, Peguillet I, Khosrotehrani K, Lantz O, Carosella ED: Skin carcinoma arising from donor cells in a kidney transplant recipient. *Cancer Res* 2005;65:1755–1760.
- 13 Murata H, Janin A, Leboeuf C, Soulier J, Gluckman E, Meignin V, Socie G: Donor-derived cells and human graft-versus-host disease of the skin. *Blood* 2007;109:2663–2665.
- 14 Janin A, Murata H, Leboeuf C, Cayuela JM, Gluckman E, Legres L, Desveaux A, Varna M, Ratajczak P, Soulier J, de The H, Bertheau P, Socie G: Donor-derived oral squamous cell carcinoma after allogeneic bone marrow transplantation. *Blood* 2009;113:1834–1840.
- 15 Hamburger J: Note préliminaire sur les greffes rénales. *Journal d'urologie* 1947. *Nephrologie* 1982;3:2–5.
- 16 Gondos A, Dohler B, Brenner H, Opelz G: Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation* 2013;95:267–274.
- 17 Wong G, Chapman JR: Cancers after renal transplantation. *Transplant Rev (Orlando)* 2008;22:141–149.
- 18 Collett D, Mumford L, Banner NR, Neuberger J, Watson C: Comparison of the incidence of malignancy in recipients of different types of organ: a UK Registry audit. *Am J Transplant* 2010;10:1889–1896.
- 19 Engels EA, Pfeiffer RM, Fraumeni JF Jr, Kasiske BL, Israni AK, Snyder JJ, Wolfe RA, Goodyrich NP, Bayakly AR, Clarke CA, Copeland G, Finch JL, Fleissner ML, Goodman MT, Kahn A, Koch L, Lynch CF, Madeleine MM, Pawlish K, Rao C, Williams MA, Castenson D, Curry M, Parsons R, Fant G, Lin M: Spectrum of cancer risk among US solid organ transplant recipients. *JAMA* 2011;306:1891–1901.
- 20 Vajdic CM, van Leeuwen MT: Cancer incidence and risk factors after solid organ transplantation. *Int J Cancer* 2009;125:1747–1754.
- 21 Kirk GD, Merlo C, P OD, Mehta SH, Galai N, Vlahov D, Samet J, Engels EA: HIV infection is associated with an increased risk for lung cancer, independent of smoking. *Clin Infect Dis* 2007;45:103–110.
- 22 Grulich AE, van Leeuwen MT, Falster MO, Vajdic CM: Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 2007;370:59–67.
- 23 Oliveira Cobucci RN, Saconato H, Lima PH, Rodrigues HM, Prudencio TL, Junior JE, Giraldo PC, Gonçalves AK: Comparative incidence of cancer in HIV-AIDS patients and transplant recipients. *Cancer Epidemiol* 2012;36:e69–e73.
- 24 Tillou X, Doerfler A, Collon S, Kleinclauss F, Patard JJ, Badet L, Barrou B, Audet M, Bensadoun H, Berthouix E, Bigot P, Boutin JM, Bouzguenda Y, Chambade D, Codas R, Dantal J, Deturmeny J, Devonec M, Dugardin F, Ferriere JM, Erauso A, Feuillu B, Gigante M, Guy L, Karam G, Lebrete T, Neuzillet Y, Legendre C, Perez T, Rerolle JP, Salomon L, Salusto F, Senechal C, Terrier N, Thuret R, Verhoest G, Petit J: De novo kidney graft tumors: results from a multicentric retrospective national study. *Am J Transplant* 2012;12:3308–3315.
- 25 Penn I: Primary kidney tumors before and after renal transplantation. *Transplantation* 1995;59:480–485.
- 26 Tsaour I, Obermuller N, Jonas D, Blaheta R, Juengel E, Scheuermann EH, Kachel HG, Karalis A, Probst M: De novo renal cell carcinoma of native and graft kidneys in renal transplant recipients. *BJU Int* 2011;108:229–234.
- 27 Rotman S, Deruaz C, Venetz JP, Chaubert P, Benhattar J, Meuwly JY, Jichlinski P, Guillou L, Moll S, Pascual M, Lemoine R: De novo concurrent papillary renal cell carcinoma and angiomyolipoma in a kidney allograft: evidence of donor origin. *Hum Pathol* 2006;37:481–487.
- 28 Alexander MP, Farag YM, Mittal BV, Rennke HG, Tullius SG, Singh AK: De novo multifocal renal cell carcinoma in the renal allograft. *Kidney Int* 2009;75:111–114.
- 29 Boix R, Sanz C, Mora M, Quer A, Beyer K, Musulen E, Gonzalez C, Bayona S, Saladie JM, Ariza A: Primary renal cell carcinoma in a transplanted kidney: genetic evidence of recipient origin. *Transplantation* 2009;87:1057–1061.
- 30 Zimmerman MA, Ghobrial RM, Tong MJ, Hiatt JR, Cameron AM, Hong J, Busuttill RW: Recurrence of hepatocellular carcinoma following liver transplantation: a review of pre-operative and postoperative prognostic indicators. *Arch Surg* 2008;143:182–188;disc. 188.
- 31 Saxena R, Ye MQ, Emre S, Klion F, Nalesnik MA, Thung SN: De novo hepatocellular carcinoma in a hepatic allograft with recurrent hepatitis C cirrhosis. *Liver Transplant Surg* 1999;5:81–82.
- 32 Croitoru A, Schiano TD, Schwartz M, Roayaie S, Xu R, Suriawinata A, Fiel MI: De novo hepatocellular carcinoma occurring in a transplanted liver: case report and review of the literature. *Dig Dis Sci* 2006;51:1780–1782.
- 33 Flemming P, Tillmann HL, Barg-Hock H, Kleeberger W, Manns MP, Klempnauer J, Kreipe HH: Donor origin of de novo hepatocellular carcinoma in hepatic allografts. *Transplantation* 2003;76:1625–1627.
- 34 Torbenson M, Grover D, Boitnott J, Klein A, Molmenti E: De novo hepatocellular carcinoma in a liver allograft associated with recurrent hepatitis B. *Transplant Proc* 2005;37:2205–2206.
- 35 Morita K, Taketomi A, Soejima Y, Ikegami T, Fukuhara T, Iguchi T, Nagata S, Sugimachi K, Gion T, Shirabe K, Maehara Y: De novo hepatocellular carcinoma in a liver graft with sustained hepatitis C virus clearance after living donor liver transplantation. *Liver Transplant* 2009;15:1412–1416.
- 36 Kita Y, Klintmalm G, Kobayashi S, Yanaga K: Retransplantation for de novo hepatocellular carcinoma in a liver allograft with recurrent hepatitis B cirrhosis 14 years after primary liver transplantation. *Dig Dis Sci* 2007;52:3392–3393.
- 37 Raviv Y, Shitrit D, Amital A, Fox B, Rosengarten D, Fruchter O, Bakal I, Kramer MR: Lung cancer in lung transplant recipients: experience of a tertiary hospital and literature review. *Lung Cancer* 2011;74:280–283.
- 38 Espinosa D, Baamonde C, Illana J, Arango E, Carrasco G, Moreno P, Algar FJ, Alvarez A, Cerezo F, Santos F, Vaquero JM, Redel J, Salvatierra A: Lung cancer in patients with lung transplants. *Transplant Proc* 2012;44:2118–2119.
- 39 Desai R, Collett D, Watson CJ, Johnson P, Evans T, Neuberger J: Cancer transmission from organ donors – unavoidable but low risk. *Transplantation* 2012;94:1200–1207.
- 40 Kauffman RJ, Chau PYK, Payne TR, Westland JC: Co-editors' introduction. *Electron Commer Res Appl* 2009;8:1–2.
- 41 Sheil AG: Donor-derived malignancy in organ transplant recipients. *Transplant Proc* 2001;33:1827–1829.
- 42 MacKie RM, Reid R, Junor B: Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. *New Engl J Med* 2003;348:567–568.
- 43 Flynn CM, Kaufman DS: Donor cell leukemia: insight into cancer stem cells and the stem cell niche. *Blood* 2007;109:2688–2692.

- 44 Spiro IJ, Yandell DW, Li C, Saini S, Ferry J, Powelson J, Katkov WN, Cosimi AB: Brief report: lymphoma of donor origin occurring in the porta hepatis of a transplanted liver. *New Engl J Med* 1993;329:27–29.
- 45 Meignin V, Devergie A, Brice P, Brison O, Parquet N, Ribaud P, Cojean I, Gaulard P, Gluckman E, Socie G, Janin A: Hodgkin's disease of donor origin after allogeneic bone marrow transplantation for myelogenous chronic leukemia. *Transplantation* 1998;65:595–597.
- 46 Thiede C, Bornhauser M, Oelschlagel U, Brendel C, Leo R, Daxberger H, Mohr B, Florek M, Kroschinsky F, Geissler G, Naumann R, Ritter M, Prange-Krex G, Lion T, Neubauer A, Ehninger G: Sequential monitoring of chimerism and detection of minimal residual disease after allogeneic blood stem cell transplantation (BSCT) using multiplex PCR amplification of short tandem repeat-markers. *Leukemia* 2001;15:293–302.
- 47 Lion T: Summary: reports on quantitative analysis of chimerism after allogeneic stem cell transplantation by PCR amplification of microsatellite markers and capillary electrophoresis with fluorescence detection. *Leukemia* 2003;17:252–254.
- 48 Beliveau BJ, Joyce EF, Apostolopoulos N, Yilmaz F, Fonseka CY, McCole RB, Chang Y, Li JB, Senaratne TN, Williams BR, Rouillard JM, Wu CT: Versatile design and synthesis platform for visualizing genomes with oligo-paint FISH probes. *Proc Natl Acad Sci USA* 2012;109:21301–21306.
- 49 Bertheau P, Plassa LF, Lerebours F, de Roquancourt A, Turpin E, Lidereau R, de The H, Janin A: Allelic loss detection in inflammatory breast cancer: improvement with laser microdissection. *Lab Invest* 2001;81:1397–1402.
- 50 Aaltonen KE, Ebbesson A, Wigerup C, Hedenfalk I: Laser capture microdissection (LCM) and whole genome amplification (WGA) of DNA from normal breast tissue – optimization for genome wide array analyses. *BMC Res Notes* 2011;4:69.
- 51 Gidlof O, Burvall S, Edvinsson L, Montelius M, Allen M, Molin M: Complete discrimination of six individuals based on high-resolution melting of hypervariable regions I and II of the mitochondrial genome. *BioTechniques* 2009;47:671–676.
- 52 He Y, Wu J, Dressman DC, Iacobuzio-Dona-hue C, Markowitz SD, Velculescu VE, Diaz LA Jr, Kinzler KW, Vogelstein B, Papadopoulos N: Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. *Nature* 2010;464:610–614.
- 53 Alkan C, Kidd JM, Marques-Bonet T, Aksay G, Antonacci F, Hormozdiari F, Kitzman JO, Baker C, Malig M, Mutlu O, Sahinalp SC, Gibbs RA, Eichler EE: Personalized copy number and segmental duplication maps using next-generation sequencing. *Nat Genet* 2009;41:1061–1067.
- 54 Verine J, Varna M, Ratajczak P, El-Bouchtaoui M, Leboeuf C, Plassa LF, Soliman H, Sandid W, Abboud I, Bousquet G, Verneuil L, Per-aldi MN, Mongiat-Artus P, Janin A: Human de novo papillary renal-cell carcinomas in a kidney graft: evidence of recipient origin with adenoma-carcinoma sequence. *Am J Transplant* 2013;13:984–992.
- 55 Lagaaij EL, Cramer-Knijnenburg GF, van Kemenade FJ, van Es LA, Buijn JA, van Krieken JH: Endothelial cell chimerism after renal transplantation and vascular rejection. *Lancet* 2001;357:33–37.
- 56 Albera C, Polak JM, Janes S, Griffiths MJ, Alison MR, Wright NA, Navaratnasah S, Poulosom R, Jeffery R, Fisher C, Burke M, Bishop AE: Repopulation of human pulmonary epithelium by bone marrow cells: a potential means to promote repair. *Tissue Eng* 2005;11:1115–1121.
- 57 Murata H, Ratajczak P, Meignin V, Groussard O, Fournier M, Socie G, Mal H, Janin A: Endothelial cell chimerism associated with graft rejection after human lung transplantation. *Transplantation* 2008;85:150–154.
- 58 Tanaka Y, Haga H, Egawa H, Okuno T, Miyagawa-Hayashino A, Tsuruyama T, Kambe M, Marusawa H, Chiba T, Manabe T: Intragraft expression of recipient-type abo blood group antigens: long-term follow-up and histological features after liver transplantation. *Liver Transplant* 2005;11:547–554.
- 59 Minami E, Laflamme MA, Saffitz JE, Murry CE: Extracardiac progenitor cells repopulate most major cell types in the transplanted human heart. *Circulation* 2005;112:2951–2958.
- 60 Woywodt A, Bahlmann FH, De Groot K, Haller H, Haubitz M: Circulating endothelial cells: life, death, detachment and repair of the endothelial cell layer. *Nephrol Dial Transplant* 2002;17:1728–1730.
- 61 Collin MP, Hart DN, Jackson GH, Cook G, Cavet J, Mackinnon S, Middleton PG, Dickinson AM: The fate of human Langerhans cells in hematopoietic stem cell transplantation. *J Exp Med* 2006;203:27–33.
- 62 Prockop DJ, Olson SD: Clinical trials with adult stem/progenitor cells for tissue repair: let's not overlook some essential precautions. *Blood* 2007;109:3147–3151.
- 63 Korbiling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, Champlin RE, Estrov Z: Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *New Engl J Med* 2002;346:738–746.
- 64 Cogle CR, Theise ND, Fu D, Ucar D, Lee S, Guthrie SM, Lonergan J, Rybka W, Krause DS, Scott EW: Bone marrow contributes to epithelial cancers in mice and humans as developmental mimicry. *Stem Cells* 2007;25:1881–1887.
- 65 Verneuil L, Varna M, Ratajczak P, Leboeuf C, Plassa LF, Elbouchtaoui M, Schneider P, Sandid W, Lebbe C, Peraldi MN, Sigaux F, de The H, Janin A: Human skin carcinoma arising from kidney transplant-derived tumor cells. *J Clin Invest* 2013;123:3797–3801.
- 66 Ingelfinger JR: Nephrogenic adenomas as renal tubular outposts. *New Engl J Med* 2002;347:684–686.
- 67 Laube GF, Haq MR, van't Hoff WG: Exfoliated human proximal tubular cells: a model of cystinosis and Fanconi syndrome. *Pediatr Nephrol* 2005;20:136–140.
- 68 Dorrenhaus A, Muller JI, Golka K, Jedrusik P, Schulze H, Follmann W: Cultures of exfoliated epithelial cells from different locations of the human urinary tract and the renal tubular system. *Arch Toxicol* 2000;74:618–626.
- 69 Mazal PR, Schaufler R, Altenhuber-Muller R, Haitel A, Watschinger B, Kratzik C, Krupitza G, Regele H, Meisl FT, Zechner O, Kerjaschki D, Susani M: Derivation of nephrogenic adenomas from renal tubular cells in kidney-transplant recipients. *New Engl J Med* 2002;347:653–659.
- 70 Poulosom R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnasah S, Jeffery R, Hunt T, Alison M, Cook T, Pusey C, Wright NA: Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 2001;195:229–235.
- 71 Moloney FJ, Comber H, O'Loirain P, O'Kelly P, Conlon PJ, Murphy GM: A population-based study of skin cancer incidence and prevalence in renal transplant recipients. *Br J Dermatol* 2006;154:498–504.
- 72 Wisgerhof HC, Edelbroek JR, de Fijter JW, Haasnoot GW, Claas FH, Willemze R, Bavinck JN: Subsequent squamous- and basal-cell carcinomas in kidney-transplant recipients after the first skin cancer: cumulative incidence and risk factors. *Transplantation* 2010;89:1231–1238.
- 73 Verneuil L, Varna M, Leboeuf C, Plassa LF, Elbouchtaoui M, Loisel-Ferreira I, Bouhidel F, Peraldi MN, Lebbe C, Ratajczak P, Janin A: Donor-derived keratinocytes in actinic keratosis and squamous cell carcinoma in patients with kidney transplant. *J Invest Dermatol* 2013;133:1108–1111.
- 74 Patel GK, Yee CL, Terunuma A, Telford WG, Voong N, Yuspa SH, Vogel JC: Identification and characterization of tumor-initiating cells in human primary cutaneous squamous cell carcinoma. *J Invest Dermatol* 2012;132:401–409.
- 75 Rehman I, Quinn AG, Healy E, Rees JL: High frequency of loss of heterozygosity in actinic keratoses, a usually benign disease. *Lancet* 1994;344:788–789.
- 76 Zong N, Xiang T, Zou Y, Chai J, Zhou JM: Blocking and triggering of plant immunity by *Pseudomonas syringae* effector AvrPto. *Plant Signal Behav* 2008;3:583–585.
- 77 Beachy PA, Karhadkar SS, Berman DM: Mending and malignancy. *Nature* 2004;431:402.
- 78 Jones TD, Eble JN, Wang M, MacLennan GT, Delahunt B, Brunelli M, Martignoni G, Lopez-Beltran A, Bonsib SM, Ulbright TM, Zhang S, Nigro K, Cheng L: Molecular genetic evidence for the independent origin of multifocal papillary tumors in patients with papillary renal cell carcinomas. *Clin Cancer Res* 2005;11:7226–7233.

- 79 Wang KL, Weinrach DM, Luan C, Han M, Lin F, Teh BT, Yang XJ: Renal papillary adenoma – a putative precursor of papillary renal cell carcinoma. *Hum Pathol* 2007;38:239–246.
- 80 Merlo LM, Pepper JW, Reid BJ, Maley CC: Cancer as an evolutionary and ecological process. *Nat Rev Cancer* 2006;6:924–935.
- 81 Campbell LL, Polyak K: Breast tumor heterogeneity: Cancer stem cells or clonal evolution? *Cell Cycle* 2007;6:2332–2338.
- 82 Clevers H: The cancer stem cell: premises, promises and challenges. *Nat Med* 2011;17:313–319.
- 83 Mengel M, Jonigk D, Wilkens L, Radermacher J, von Wasielewski R, Lehmann U, Haller H, Mihatsch M, Kreipe H: Chimerism of metanephric adenoma but not of carcinoma in kidney transplants. *Am J Pathol* 2004;165:2079–2085.
- 84 Kim JK, Carmody IC, Cohen AJ, Loss GE: Donor transmission of malignant melanoma to a liver graft recipient: case report and literature review. *Clin Transplant* 2009;23:571–574.
- 85 de Boer M, Vink A, van de Graaf EA: Non-small cell lung carcinoma of donor origin after bilateral lung transplantation. *Lung Cancer* 2012;76:259–261.
- 86 Seda Neto J, Macedo C, Jaffe R, Mazariegos GV, Sindhi R, Bond GJ, Abu-Elmagd K, Reyes J: Carcinoma of donor origin after liver-intestine transplantation in a child. *Pediatr Transplant* 2005;9:244–248.
- 87 Smith MJ, van Cleef PH, Schattenberg AV, van Krieken JH: The origin of epithelial neoplasms after allogeneic stem cell transplantation. *Haematologica* 2006;91:283–284.
- 88 Aractingi S, Sibilia J, Meignin V, Launay D, Hachulla E, Le Danff C, Janin A, Mariette X: Presence of microchimerism in labial salivary glands in systemic sclerosis but not in Sjögren's syndrome. *Arthritis Rheumat* 2002;46:1039–1043.
- 89 Chiang S, Yip S, Betensky RA, Batten JM, Misdrabi J, John Iafrate A: Identification of tissue contamination by polymorphic deletion probe fluorescence in situ hybridization. *Am J Surg Pathol* 2012;36:1464–1471.