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Risk of virus and non-virus related malignancies following immunosuppression in a cohort of liver transplant recipients. Italy, 1985–2014

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Abbreviations: CI: confidence interval; EBV: Epstein-Barr virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HPV: human papilloma virus; IQR: interquartile range; KS: Kaposi's sarcoma; KSHV: Kaposi sarcoma herpes virus; LT: liver transplantation; NHL: non-Hodgkin lymphomas; PY: person-year; SIR: standardized incidence ratio

Additional Supporting Information may be found in the online version of this article.

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This cohort study assessed, in Italy, the overall pattern of risk of *de novo* malignancies following liver transplantation (LT). The study group included 2,832 individuals who underwent LT between 1985 and 2014 in nine centers all over Italy. Person–years (PYs) at cancer risk were computed from 30 days after LT to the date of cancer diagnosis, to the date of death or to the end of follow-up. Excess cancer risk, as compared to the general population, was estimated using standardized incidence ratios (SIRs) and 95% confidence intervals (CIs). During 18,642 PYs, 246 LT recipients developed 266 *de novo* malignancies, corresponding to a 1.8-fold higher cancer risk (95% CI: 1.6–2.0). SIRs were particularly elevated for virus-related malignancies, including Kaposi's sarcoma (SIR = 53.6, 95% CI: 30.0–88.5), non-Hodgkin lymphomas (SIR = 7.1, 95% CI: 4.8–10.1) and cervix uteri (SIR = 5.4, 95% CI: 1.1–15.8). Among virus-unrelated malignancies, elevated risks emerged for head and neck (SIR = 4.4, 95% CI: 3.1–6.2), esophagus (SIR = 6.7, 95% CI: 2.9–13.3) and adrenal gland (SIR = 22.9, 95% CI: 2.8–82.7). Borderline statistically significant elevated risks were found for lung cancer (SIR = 1.4, 95% CI: 1.0–2.1) and skin melanoma (SIR = 2.6, 95% CI: 1.0–5.3). A reduced risk emerged for prostate cancer (SIR = 0.1, 95% CI: 0.0–0.5). These findings underline the need of preventive interventions and early detection of malignancies, specifically tailored to LT recipients.

What's new?

Liver transplantation often requires long-term immunosuppressive therapy, which increases the risk of certain infections and malignancies. The extent to which chronic immunosuppressant use impacts cancer risk following liver transplantation, however, remains unclear. In this multicenter cohort study in Italy, liver transplant recipients had an overall 1.8-fold higher cancer risk compared with the general population. Risk was elevated for virus-related malignancies, as well as for several cancers not associated with viral infections, including cancers of the head and neck, esophagus, and adrenal gland. The findings support further investigation into the prevention and early detection of cancer in liver transplant recipients.

Introduction

Liver transplantation (LT) is the treatment of choice for patients with end-stage liver disease, acute liver failure or hepatocellular carcinoma. In Italy, a total of 17,174 liver transplants were performed between 2000 and 2016, accounting for approximately 1,000 LTs per year.[1] Although survival outcomes following LT have improved substantially over the last decades, the chronic use of immunosuppressive therapy remains associated with an increased risk of opportunistic diseases, particularly infections and malignancies.[2]

De novo malignancies represent a major adverse outcome of LT, with cumulative incidences showing wide variations according to geographic area, period and study duration.[3,4] Previous investigations have found that LT recipients had an overall two- to threefold increased risk of cancer than the general population of the same age and sex.[2,3,5,6] Particularly, elevated risks were registered for virus-related cancers, including non-Hodgkin lymphomas (NHL) (Epstein-Barr virus [EBV]), Kaposi's sarcoma (KS) (human herpes virus-8 [KSHV]) and liver cancer (hepatitis B and C viruses [HBV and HCV, respectively]). In addition, certain malignancies with non infectious causes, such as lung, skin or head and neck cancers were also reported to be elevated among LT recipients in comparison with the corresponding general population.[3,7]

As seen in the HIV infection and AIDS scenery,[8] the investigation of the pattern of cancer risk associated with iatrogenic post-transplant immunosuppression may help to clarify the role of the immune system in relationship between chronic viral infections, other known risk factors and the development of malignancies. Although several large studies have been conducted in the United States,[3] Asia[9] or Northern Europe,[6,10] relatively few investigations have quantified, in Southern Europe, the excess cancer risk among LT recipients as compared to the general population. Our study group started investigating this issue in Italy few years ago, and some articles have been already published on specific aspects of *de novo* malignancies after LT.[11–13]

In our prospective study, we assessed the overall pattern of *de novo* malignancies following LT to quantify the excess risk of cancer among Italian LT recipients, as compared to the corresponding general population.

Materials and Methods

A cohort study was conducted using clinical and epidemiological data collected among 3,121 individuals who underwent LT in 9 centers from all over Italy, between 1985 and 2014. Specifically excluded from this analysis were patients who met at least one of the following conditions: (*i*) a previous organ transplant ($n = 23$); (*ii*) a follow-up shorter than 30 days after LT ($n = 232$); (*iii*) missing information on date of birth ($n = 4$); (*iv*) age at LT below 18 years ($n = 28$) and (*v*) a cancer diagnosis within 30 days after LT ($n = 2$). Therefore, our study group consisted of 2,832 LT recipients.

In each centre, trained staff retrieved appropriate information from clinical charts and checked data for accuracy and

completeness. Data were collected on standardized forms that included personal information (*e.g.,* sex, age at transplant, area of origin and residence), transplant information (*e.g.,* transplant centre, date of LT, underlying disease and donor status) and data about follow-up and vital status. Cancer diagnoses were ascertained as a result of clinical follow-up. They were all histologically confirmed and coded according to the International Classification of Diseases and Related Health Problems, 10th revision (ICD-10).

Person–years (PYs) at risk of cancer were computed as the time elapsed from 30 days after LT to the date of cancer diagnosis, to the date of death or to the end of follow-up, whichever came first. We excluded from the analysis the first 30 days of follow-up to eliminate those cancers that were prevalent at transplantation but not diagnosed because of borderline clinical significance. After a cancer diagnosis, patients no longer contributed to the determination of person–time at risk of that specific cancer type. However, these patients continued to be at risk of other cancer types in the specific analysis. LT recipients with a history of cancer—other than hepatocellular carcinoma—before transplantation were not considered eligible to contribute to person–time at risk of that specific cancer type. On the other hand, those patients with pre-LT hepatocellular carcinoma contributed to the determination of person–time at risk of *de novo* liver cancer.

Standardized incidence ratios (SIRs) were computed as the ratio between the observed and the expected number of cancer cases.[14] For patients diagnosed with more than one malignancy within the same ICD-10 group (*e.g.,* colon-rectum ICD-10 codes: C18–20; head and neck: C00–14, C30–32 and all: C00–97), only the first one was considered. The expected number of cases was calculated according to the incidence rates among the Italian general population obtained from all Italian cancer registries, as published in Cancer Incidence in Five Continents, vol. VII (for the period up to 1992), vol. VIII (1993–1997), vol. IX (1998–2002), vol. X (2003–2007)[15] or from ITACAN (from 2008 thereafter).[16] SIRs were standardized by 5-year age group, sex, area of residence and calendar period. Corresponding 95% confidence intervals (CIs) were computed assuming a Poisson distribution.[14] SIRs were presented only for cancer sites with at least two observed cases. SIRs with a $p < 0.05$ were marked with *, those with a $p < 0.01$ with **, and those with a $p < 0.00125$ (Bonferroni correction, based on 40 comparisons) with ***. To estimate cumulative cancer incidence by time since LT and cancer type, a competing risk approach with nonparametric estimators was used,[17] accounting for death as a competing event.

All statistical analyses were performed using the software SAS Version 9.4 (SAS Institute Inc., Cary, NC).

Results

The 2,832 LT recipients were followed-up for a total of 18,642 PYs of observation (the median length of follow-up was 5.4 years; interquartile range, IQR: 2.4–10.0 years), with a median age at transplant of 53 years (Table 1). The majority of LT recipients were males (74.6%), underwent LT between 2006 and 2014 (39.3%), resided in southern Italy (50.0%) and the transplanted liver was usually from a deceased donor (96.1%). Among the study subjects 50% had a history of HCV infection, whereas HBV infection was documented in 42.1% of LT recipients. Information on smoking history was available for 1,359 (48%) study subjects of whom 841 had a history of smoking. Alcohol abuse was reported by 27.4% of LT recipients.

During the period of observation, 246 LT recipients developed 266 *de novo* malignancies (the median age at cancer diagnosis was 59.8 years; IQR: 52.5–64.8 years). Cumulative incidence estimates of *de novo* malignancies within the first 10 years after LT, taking into account death as a competing event, are illustrated in Figure 1. The cumulative incidence for all cancers other than non-melanoma skin cancers increased steadily over the follow-up period (5.2% at 5 years and 8.2% at 10 years). The 5- and 10-year cumulative risks were 0.7% and 1.5% for NHL, and 0.5% and 0.7% for KS, respectively. Among virus-unrelated malignancies, the cumulative incidences at five and 10 years post-transplant were 0.6% and 1.3% for lung cancers, and 1.1% and 1.4% for head and neck cancers, respectively. Despite the continuous improvements in surgical techniques and management of immunosuppression, no differences in cumulative cancer incidence emerged according to the calendar period at LT (1985–2001, 2002–2005 and 2006–2014) (data not shown).

The age-specific incidence rates of *de novo* malignancies observed in LT recipients were consistently higher than those in the general population, even though these differences decreased with increasing age (Fig. 2).

Table 2 shows the observed and the expected numbers of cancer cases with the corresponding SIRs. When considering all cancer types a 1.8-fold higher cancer risk in LT recipients was found as compared to the general population (95% CI: 1.6–2.0, Table 2). After the exclusion of non-melanoma skin cancers, the SIR was 1.7 (95% CI: 1.5–1.9). The SIRs were particularly elevated for virus-related malignancies, including KS (SIR = 53.6, 95% CI: 30.0–88.5), NHL (SIR = 7.1, 95% CI: 4.8–10.1) and cervix uteri (SIR = 5.4, 95% CI: 1.1–15.8). Among virus-unrelated malignancies, elevated excess risks emerged for head and neck (SIR = 4.4, 95% CI: 3.1–6.2), esophagus (SIR = 6.7, 95% CI: 2.9–13.3) and adrenal gland (SIR = 22.9, 95% CI: 2.8–82.7). Borderline statistically significant elevated risks were found for bronchus and lung (SIR = 1.4, 95% CI: 1.0–2.1) and for skin melanoma (SIR = 2.6, 95% CI: 1.0–5.3). Conversely, a significantly reduced risk was noted for prostate cancer (SIR = 0.1, 95% CI: 0.0–0.5). SIRs for cancer sites with one or zero observed cases are presented in Supporting Information Table S1.

The SIRs were generally higher among females (Supporting Information Table S2). In particular, females reported higher excess risks than males for KS (SIR = 193.2, 95% CI: 52.6–494.6 in females; SIR = 42.7, 95% CI: 21.3–76.4 in males), for esophagus (SIR = 30.5, 95% CI: 3.7–110.1 in females; SIR = 5.3, 95% CI: 2.0–11.6 in males) and for head

Table 1. Distribution of 2,832 patients who underwent liver transplantation by selected characteristics

	All patients, *N* (%)
Sex	
Male	2,113 (74.6)
Female	719 (25.4)
Age at transplant (years)	
18–44	623 (22.0)
45–54	958 (33.8)
≥55	1,251 (44.2)
Median (IQR)	53 (46–59)
Calendar year at transplant	
1985–2001	843 (29.8)
2002–2005	875 (30.9)
2006–2014	1,114 (39.3)
Area of residence	
Northern Italy	699 (24.7)
Central Italy	682 (24.1)
Southern Italy	1,415 (50.0)
Abroad	21 (0.7)
Unknown	15 (0.5)
Status of the donor	
Alive	109 (3.9)
Deceased	2,723 (96.1)
History of HBV infection	
No	1,640 (57.9)
Yes	1,192 (42.1)
History of HCV infection	
No	1,412 (49.9)
Yes	1,420 (50.1)
History of alcohol abuse	
No	2,056 (72.6)
Yes	776 (27.4)
History of smoking	
No	518 (18.3)
Yes	841 (29.7)
Unknown	1,473 (52.0)
Vital status	
Alive	2,086 (68.7)
Deceased	746 (26.3)
Patients with *de novo* malignancies after transplant	
No	2,586 (91.3)
Yes	246 (8.7)
No. of de novo malignancies after transplant	
1	*227 (92.3)*
2	*18 (7.3)*
3	*1 (0.4)*
Follow-up (years)	
Median (IQR)	5.4 (2.4–10.0)
Total person–years	18,641.5

Abbreviations: IQR: interquartile range; HBV: hepatitis B virus; HCV: hepatitis C virus.

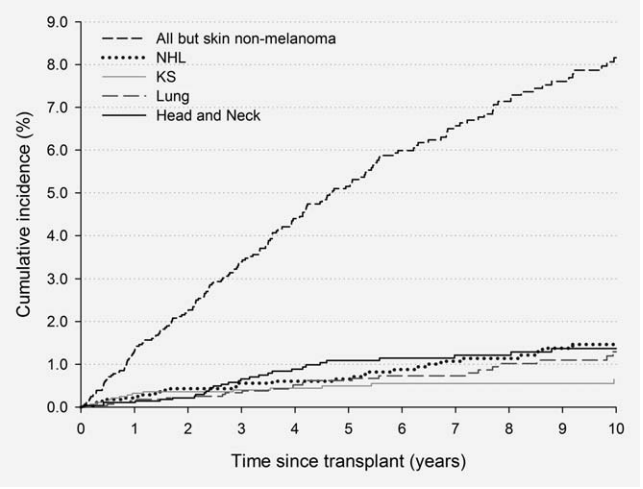

Figure 1. Cumulative cancer incidence by time since liver transplantation and cancer type. Abbreviations: KS: Kaposi's sarcoma; NHL: non-Hodgkin lymphoma.

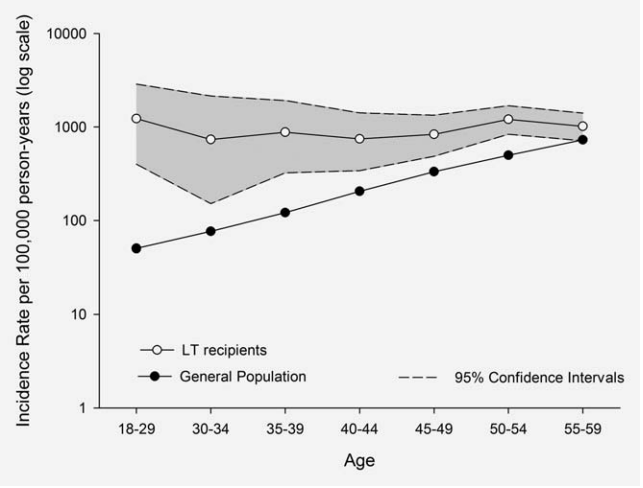

Figure 2. Age-specific incidence rates for *de novo* malignancies observed in liver transplant (LT) recipients and in the Italian general population.

and neck (SIR = 12.4, 95% CI: 4.0–28.8 in females; SIR = 4.0, 95% CI: 2.7–5.7 in males).

Discussion

This cohort study provided estimates of the cancer excess risk among Italian LT recipients as compared to the

Table 2. SIRs and 95% CIs for *de novo* malignancies in liver transplant recipients

Type/site	ICD-10 codes	Total		SIR (95% CI)
		Obs.	Exp.	
Virus-related malignancies				
Non-Hodgkin lymphoma	C82–85, C96	31	4.4	7.1 (4.8–10.1)***
Kaposi's sarcoma	C46	15	0.3	53.6 (30.0–88.5)***
Liver	C22	6	5.5	1.1 (0.4–2.4)
Cervix uteri	C53	3	0.6	5.4 (1.1–15.8)*
Hodgkin lymphoma	C81	2	0.6	3.5 (0.4–12.6)
Virus-unrelated malignancies				
Head and neck	C00–14, C30–32	34	7.7	4.4 (3.1–6.2)***
Bronchus and lung	C34	28	19.4	1.4 (1.0–2.1)
Colon–rectum	C18–20	21	15.9	1.3 (0.8–2.0)
Bladder	C67, D09.0, D30.3, D41.4	9	11.4	0.8 (0.4–1.5)
Esophagus	C15	8	1.2	6.7 (2.9–13.3)***
Stomach	C16	7	5.7	1.2 (0.5–2.5)
Skin melanoma	C43	7	2.7	2.6 (1.0–5.3)*
Thyroid gland	C73	5	2.3	2.2 (0.7–5.0)
Breast female	C50	4	8.6	0.5 (0.1–1.2)
Kidney	C64	4	4.2	1.0 (0.3–2.5)
Pancreas	C25	3	3.3	0.9 (0.2–2.6)
Leukemia	C91–95	3	2.9	1.0 (0.2–3.0)
Prostate	C61	2	14.0	0.1 (0.0–0.5)***
Testis	C62	2	0.4	5.2 (0.6–18.7)
Adrenal gland	C74	2	0.1	22.9 (2.8–82.7)**
Unspecified sites	C76–C80	5	1.9	2.6 (0.8–6.0)
Skin non-melanoma	C44	50	18.3	2.7 (2.0–3.6)***
All lymphohematopoietic malignancies[1]	C81–96	37	9.6	3.8 (2.7–5.3)***
All solid tumors but skin non-melanoma[1,2]		149	112.6	1.3 (1.1–1.6)***
All but skin non-melanoma[1,2]		199	117.5	1.7 (1.5–1.9)***
All[1,2]		246	136.5	1.8 (1.6–2.0)***

[1]It includes sites/types with <2 observed cases, which were not shown in table.
[2]The sums can exceed the total because some patients were diagnosed with more than one malignancy. For patients diagnosed with more than one malignancy within the same ICD-10 group (*e.g.,* colon-rectum ICD-10 codes: C18–20; head and neck: C00–14, C30–32; all: C00–97), only the first one was considered. *$p < 0.05$, **$p < 0.01$, ***$p < 0.00125$ (corresponding to the Bonferroni level of statistical significance based on 40 comparisons).
Abbreviations: Exp: expected number of cancer cases; Obs: observed number of cancer cases; SIR: standardized incidence ratio; CI: confidence interval.

corresponding general population. About 9% of LT recipients developed cancer over the follow-up period, equivalent to a 1.8-fold higher cancer risk. In addition to the elevated risks of virus-related malignancies, the study also documented statistically significant elevated risks of several cancers not known to be associated with chronic viral infections.

Overall, the elevated cancer risk found among LT recipients—with respect to the general population—was in line with those described in previous studies carried out in other industrialized countries (SIRs $\approx$ 2).[3,9] As expected, particularly elevated risks emerged for cancers associated to viral infections, which LT recipients are prone to develop in the setting of immunosuppression. The very high excess risk registered for KS, particularly in women (SIR = 193), was consistent with the results from studies performed in the Mediterranean area, where KS often represents the major group of cancers following organ transplantation. Indeed, the increased risk of KS is attributable to the high prevalence rates of infection with KSHV documented in some Mediterranean countries, particularly, in Italy.[18,19] Among virus-related malignancies, NHL was the most common cancer diagnosed after LT. In transplant recipients, poor immune control of EBV has been linked to the high incidence of NHL and post-transplant lymphoprolipherative diseases. In

accordance with other studies, we found a high excess risk of NHL.[2,3,7] Furthermore, although the analyses were based on small number of cases, there was an indication of excess risk of Hodgkin's lymphoma. Conversely, results from previous studies did not report a high excess risk of cervical cancer.[3,20,21] To this regard, immunosuppression seems to be important in facilitating Human Papilloma Virus (HPV) replication and/or persistence by disrupting the local immunosurveillance, but it apparently has a lower effect in promoting progression to cancer.[22] Regarding *de novo* liver cancer, which is strongly associated to infections with HCV and HBV, we did not find a high risk in line with other studies.[2,10] On the contrary, a large cohort study from United States evidenced that LT recipients had a strongly elevated risk of liver cancer compared to the general population.[3] However, these differences seem attributable to the fact that, among LT recipients, elevated incidence of liver cancer within the first years after transplantation may be due to prevalent cancer cases from the explanted liver or recurrence of the underlying malignancy, which was not considered in our analysis. As far as HCV infection is concerned, the scenario will probably change further in the future due to the positive effect of direct-acting antivirals on HCV recurrence after transplant.[23]

The spectrum of virus-unrelated malignancies, for which we found excess risks, and the magnitude of SIRs, were in line with our previous investigations[12,13] and with other studies conducted in high-income countries.[2,3,9] Our findings confirmed prior evidence of an excess risk of cancers associated with unhealthy behaviors, such as tobacco smoking, alcohol abuse or sun exposure. In this cohort, the most commonly detected virus-unrelated malignancies were head and neck cancers ($n = 34$), followed by lung ($n = 28$) and colorectal cancers ($n = 21$). Our estimate of head and neck cancer risk was similar to the findings from other European cohorts,[7,24,25] whereas a non-significant, increased risk was found in Canadian[26] and Asian studies.[9,27] Notably, as shown in our previous work[13] that focused on alcohol-related cancers alone, an elevated excess risk of head and neck and esophageal cancers emerged among LT recipients, particularly, among those patients with a background of alcoholic liver diseases. We also observed a high excess risk of lung cancer in line with previous studies.[3,24] These results are consistent with the evidence of the carcinogenic properties of alcohol and smoking in immunocompetent individuals.[28,29] Non-melanoma skin cancer was the most common malignancy diagnosed among the cohort members. As for immune competent individuals, prior exposure to solar ultraviolet radiation is a principal risk factor, with squamous cell carcinomas most likely to occur at sun-exposed areas and in transplant recipients with a history of high sun exposure.[22] In our LT population, of 50 non-melanoma skin cancer cases, 52% were basal cell carcinoma, 32% were squamous cell carcinoma and 16% were other/not otherwise specified. We could not evaluate separately the excess risk of squamous cell and basal cell skin cancers as compared to the general population, because population cancer registries do not appropriately differentiate these tumors.

Prostate cancer was the only cancer site for which a statistically significant SIR below one was observed. While the incidence of prostate cancer may be reduced among LT recipients, thanks to pre-transplant screening, we found no evidence of a reduced risk of breast cancer. Our findings did not reveal an excess risk of colorectal cancer. Although, prior studies have produced mixed results,[2,7,30] a high excess risk of colorectal cancer has been well established in patients with primary sclerosing cholangitis.[7,31] To the best of our knowledge, this is the first study showing an increased risk of adrenal gland cancer after LT in comparison with the general population, although the role of chance cannot be excluded.

Some study limitations have to be acknowledged. First, the lack of completeness in the ascertainment of *de novo* cancer cases was possible as cancer diagnoses were registered on the basis of clinical records. Although we could not perform a linkage with population-based cancer registries for all LT recipients, the strict clinical follow-up of these patients is likely to ensure the completeness of cancer reporting. Second, smoking history was available only in 48% of study subjects, as this information is not regularly collected in Italian LT transplant centers. However, the role of smoking in the etiology of smoking-related cancers is well known and this lack of completeness had limited impact on our aim of quantifying the excess risk of cancer among Italian LT recipients, particularly in the analysis of virus-related cancers. Third, the study has limited power to detect the excess risk of specific subtypes, thus, calling for caution in interpretation of results. Moreover, the issue of multiple comparisons may lead to accept false positive associations due to chance. However, this has rarely been taken into account in the literature focused on post-transplant cancers. For this reason, we simply marked those SIRs with an exact p value below the Bonferroni level of statistical significance. Despite these limitations, our study is the largest cohort to provide an overall picture of cancer risk among LT recipients in a southern European population. The length of follow-up and the geographic heterogeneity of the cohort represent important strengths of our study.

New areas of research are also needed, in view of the decreasing cancer risk after LT observed in a Nordic European population over 3 decades[32] and the need to focus on populations rarely investigated like the LT transplanted because of non-alcoholic fatty liver disease or non-alcoholic steatohepatitis.[33–36]

In conclusion, our findings underline the need of monitoring the burden of both virus-related and virus-unrelated malignancies among LT recipients, as they are at a higher risk of developing *de novo* malignancies than the corresponding general population. The elevated risk emerged for a broad range of malignancies following LT should encourage further interventions of prevention and early detection of cancer

tailored to this population. Thus, LT recipients who are chronic carriers of infections with known oncogenic viruses (such as EBV, KSHV and HPV) deserve an active monitoring particularly during the first couple of years after transplant, when the highest dose of immunosuppressive drugs are prescribed. Moreover, regular dermatological monitoring, minimizing sun exposure and early treatment of premalignant lesions should be encouraged to reduce skin cancer risk, especially for subjects at higher risk. Furthermore, prevention strategies aimed at the general population such as adherence to existing screening programs, smoking and alcohol drinking cessation, a correct diet and physical activity, should be especially emphasized among the LT recipient population.

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