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Immunological aspects of neoplastic diseases

V. Radhakrishna, M.D.*

The immunological approach to neoplastic disease is gaining favor because malignant tumors are associated with immunodeficient states. Mesenchymal tumors have been seen in renal allograft recipients. The discovery of carcinoembryonic antigen, alfa-fetoprotein and other protein antigens associated with malignant diseases are other factors. Spontaneous regression of malignant neoplasm has been attributed to an effective immunologic process in the host. Identification of tumor specific antigens may explain these spontaneous tumor regressions.

It may not be too optimistic to expect tumor vaccine of some sort within the next few years.

B.C.G. and other nonspecific immunostimulants are under investigation for treatment of malignant tumors. Transfer factor has been extensively used in the last 20 years and seems to be useful in certain types of tumors. Immune RNA may be a reasonable approach to adoptive transfer of immunity.

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AROUND 1950 the concept developed that cancer might be due to the deletion of some components necessary for maintaining normal relationship between cells in a tissue. The term "surveillance" was first coined by Burnet¹ based on the occurrence of spontaneous tumors in renal transplantation patients who were receiving long-term immunosuppressive therapy. He hypothesized that a clone of malignant cells arises at regular intervals in all individuals, but cells are rejected as allografts unless there is some unspecified defect in immune response. This certainly is a stimulating idea but it is still not clear how we are protected from oncogenic events by the immune mechanism.

Tumor antigens

Of all the parts that theoretically may be transformed in malignancy, the cell surface may be the most important. The discovery of *tumor specific antigens* (TSA) on the cell surface may permit early diagnosis.² Although TSA are probably not only confined to the surface, they are most readily detected on intact cells. Moreover, TSA on the surface are sensitive to the immune response which may result in tumor destruction.

(a) *Tumor specific antigens.* Immunological methods which are specific, sensitive and relatively inexpensive may soon be the best means to diagnose cancers that produce distinctive antigens. Unfortunately, all cancers are not alike in this respect, so only some of them may be detected by immunological means. In chemically induced tumors, the new tumor antigens are different for each tumor. Even two tumors induced in the same animal by the same carcinogen appear to be antigenically different from each other (Figure 1).

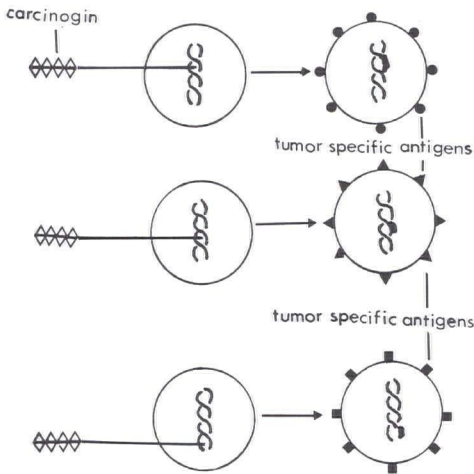


Figure 1
Tumor antigens induced by chemical carcinogens. When cells of identical genetic background are transformed with the same chemical carcinogen, each new tumor has its own antigenic specificity which is not shared with other tumors. (Immunology monograph by UpJohn Co. 1975)

On the other hand, animal tumors induced by viruses develop a new antigen that is common to all the tumors induced by the same virus, even though the tissue origins of tumors are different (Figure 2). Both DNA and RNA viruses can induce these antigen specific tumors. Among DNA viruses that can code for new antigens are herpes viruses, adenoviruses, polyoma and papilloma

viruses. Two groups of viruses have been consistently associated with naturally occurring cancer—oncornaviruses containing single stranded RNA, and herpes virus with double stranded DNA.

The most important characteristic of oncornaviruses is that they contain DNA polymerase (Reverse Transcriptase). This enzyme reverses the usual direction of information (DNA → RNA) so that DNA is produced for RNA template. This DNA intermediate is then integrated with the host genome, causing neoplastic transformation by some unknown mechanism.

In man, Epstein-Barr virus (herpes group) has been associated with Burkitt's lymphoma, nasopharyngeal carcinoma and Hodgkin's disease. One of the most extensively studied virus-associated cancers is carcinoma of cervix, where there is a constant elevation of herpes simplex Type II virus antibodies in patients.

There is good circumstantial evidence that RNA and DNA viruses cause cancer.³ Whether or not cancer develops following exposure to these viruses probably depends on the immunologic response of the infected animal. Spontaneously occurring tumors were long considered to lack TSA. The exact nature of TSA is not known but it may be part of virus particles or component of tumor cells.¹

(b) *Carcinoembryonic antigen (CEA).* In 1965 Gold demonstrated the presence of a glycoprotein antigen in patients with cancer of the colon.⁴ Subsequently this antigen was shown to be present in up to 97% of such patients with metastasis.⁵ Further studies revealed that the levels of CEA vary with the stage of the disease. In three series of studies the CEA level varied from a low of 19% to 40% in patients with localized disease to a high of 100% in patients with disseminated disease, especially of the liver.⁵⁻⁷ But the greatest limitation of screening by CEA is its nonspecificity for cancer of the colon. Many other types of cancers have elevated levels

Immunological aspects of neoplastic diseases

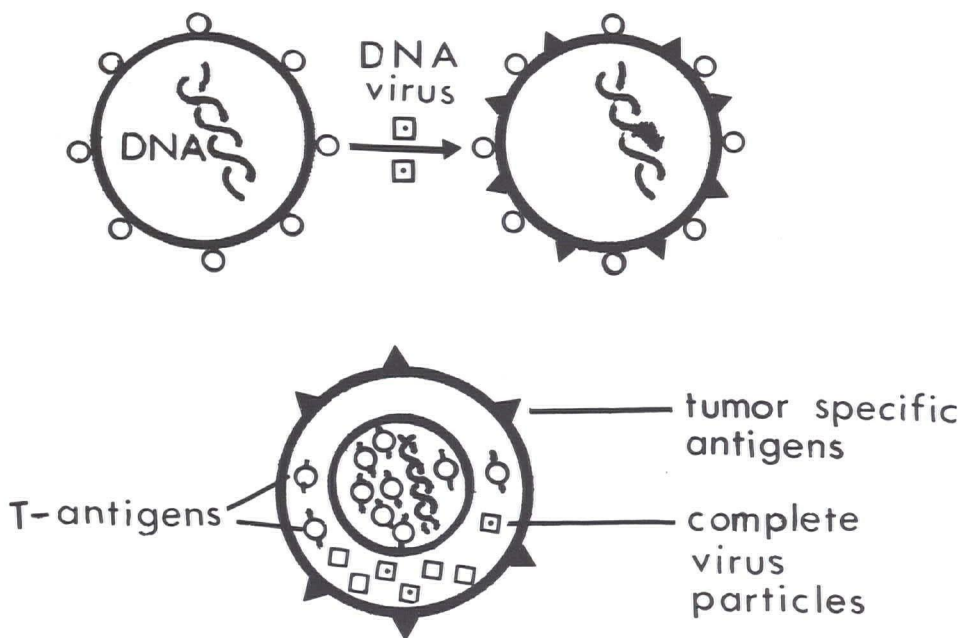


Figure 2

Three new antigens in a virus transformed cell. Virus capsid antigen and new virus particles may be found in the malignant transformed cell recently infected and destined to undergo lysis. (Immunology monograph by UpJohn Co. 1975)

of CEA. For example, CEA is elevated in as many as 90% of patients with pancreatic carcinoma⁵ (Table I). Nor is elevated CEA limited to malignant diseases. Many benign disorders also have higher levels of CEA (Table II) especially liver disease, alcoholic pancreatitis and benign obstructive jaundice.⁵ Most patients with inactive ulcerative colitis have normal levels of CEA. Elevated levels tend to correlate with exacerbation of activity.⁶ Screening for CEA levels is most useful in postoperative follow-up of patients with colonic carcinoma. Many investigators have confirmed that elevated CEA levels fell to normal after resection of tumor.⁶ Postoperative serial CEA determinations may identify metastases before clinical evidence of spread of the disease.⁶

Generally speaking, higher levels of CEA are associated with poorer prognosis. However, several investigators confirm that

normal levels of CEA do not preclude primary, metastatic or recurrent disease.^{6,7}

(c) *Alphafetoprotein (AFP)*. The association of AFP with primary liver cancer was observed more than ten years ago.⁸ Though originally thought to be specific for primary hepatoma, AFP has been found to be elevated in several other conditions, including some normal individuals⁹ (Table III). In primary hepatoma up to 70% of patients have elevated AFP; 80% of patients with teratocarcinoma and a small percentage of patients with pancreatic, gastric, colonic carcinoma also have elevated AFP levels.^{8,30}

(d) *Other Tumor Antigens*

i) *Alfa₂-H Feto Protein* is immunologically identical to ferritin and is present in the liver and serum of the human fetus.¹⁰ Though initially detected in about 60% of

Radhakrishna

Cancer	Incidence of Positive Assays	
	Percentage	No. of Cases
Colon & Rectum	73	316
Pancreas	92	52
Liver	67	18
Breast	52	159
Cervix	42	45
Endometrium	27	11
Ovary	35	20
Testis	57	29
Prostate	40	93
Kidney	35	26
Bladder	33	106
Neuroblastoma	100	6
Leukemia	22	27
Lymphoma	25	14
Bronchus	72	90

Compilation of reported observations made with Gold, Hansen or Todd assays from reports by Zamcheck, Lo Gerfo, Reynoso, and Laurence. Modified from Laurence and Neville.

patients with primary hepatoma, 50% of patients with various malignancies and 20-30% of certain benign disorders have elevated alfa₂ — H fetoprotein levels. In children hepatic Alfa₂ — HFP appears more specific (80% positivity) despite 10% false positive results.¹⁰

ii) *Beta -S-fetoprotein* and *Gamma -S-fetoproteins* are found in a wide variety of malignant and nonmalignant conditions. Specific data regarding these antigens is lacking at this time.

iii) *Fetal sulfoglycoprotein antigen (FSA)*. In initial studies 75 of 78 patients with gastric carcinoma had elevated levels of FSA in their gastric juice.¹¹ But there was no fall in FSA levels even after complete removal. FSA is elevated in 10-15% of benign gastric ulcers.

Disease	Incidence of Positive Assays	
	Percentage	No of Cases
Alcoholic cirrhosis, severe	45	88
Alcoholic pancreatitis	43	42
Pulmonary tuberculosis	38	21
Chronic bronchitis and emphysema	25	63
Inflammatory bowel disease	21	95
Diverticulitis and peptic ulcer	21	33
Colorectal polyps	9	67
Prostatic - hypertrophy	7	30
Breast - fibroadenosis	7	70
Gastric atrophy	5	19

Norman Zamchek M. D. and Gabor Pusztaszeri M. D. ; CEA, AFP and other potential tumor makers: CA-Cancer Journal for Clinicians, vol. 25, No. 4 July/August 1975.

Immunological aspects of neoplastic diseases

iv) *Carcinoplacental alkaline phosphatase* is a nonspecific tumor marker and is found in highest frequency in ovarian (45%), testicular (40%) and pancreatic (30%) cancers.¹²

v) *Oncofetal antigens (OFA)*. In preliminary studies 29 of 30 patients with carcinoma of the pancreas had high levels of oncofetal antigens. Further work is needed to confirm the importance of this antigen.¹³

Evidence for a successful immunological surveillance

That immunological factors influence the natural course of certain tumors in man may

be indirectly observed by comparing the incidence of neoplasia in immunosuppressed and normal populations.

There is sufficient evidence that impairment of immunological capacity increases the susceptibility to cancer and accelerates its growth¹⁴ (Table IV). The use of immunosuppressive agents (particularly anti-lymphocytic serum) to prevent rejection of a grafted organ in man has provided further support for the existence of immunological surveillance that rids the body of incipient neoplastic cells. The incidence of neoplasia in patients with renal allograft transplant has been 20 to 80 times higher than in general population.¹⁴ Most of these tumors are lymphoreticular, epithelial or anaplastic in nature.

Table III
Serum Alpha-Fetoprotein in Patients with Cancer

Diagnosis	No. of samples assayed	Percent over 40 ng./ml.
Hepatocellular carcinoma	180	72
Testicular teratocarcinoma	101	75
Pancreatic carcinoma	44	23
Gastric carcinoma	91	18
Colonic carcinoma	193	5
Bronchogenic carcinoma	150	7
Breast carcinoma	55	0
Nonhepatic benign lesions	300	0.3
Normal controls over 1 year of age	210	0

From: Waldmann, T.A., and McIntire, K.R.: The use of radio-immunoassay for alpha-fetoprotein in the diagnosis of malignancy. *Cancer* 34: 1510-1515, 1974.

<p align="center">Table IV The Incidence of Primary Cancer in Immunologically Deficient and Renal Transplant Patients</p>		
Disease	Percent Incidence of Cancer	Tumor Type
Sex-linked agammaglobulinemia* (Brutons)	5 (10,000 x normal)	Acute lymphatic leukemia
Severe combined immunodeficiency	5	Lymphoreticular
Wiskott-Aldrich	10	Lymphoreticular
Common variable immunodeficiency	10	Lymphoreticular & carcinomas
Isolated IgA	-	Adenocarcinoma of stomach (one case)
Ataxia telangiectasia	10	Lymphoreticular, sarcoma, carcinoma
Renal transplant patients **	1.26(190/15,000) (25 x normal)	Epithelial (62 percent) Mesenchymal (38 percent)
<p>*From Richard A. Gatti and Robert A. Good **From Olga Jonasson and Israel Penn and Thomas E. Starzl</p>		

Burnet¹ has studied denovo malignancies in 42 renal homograft recipients and came out with certain interesting points; viz:

- a) Patients tended to be of younger age (8-54 years) with a mean of 33 years.
- b) All had immunosuppression with azothiaprism and prednisone.
- c) 21 of 42 cases (50%) had mesenchymal tumors (most commonly reticulum cell sarcoma).
- d) Highly malignant tumor tends to appear much earlier after transplantation than the relatively low grade malignancies of skin, lips, etc.
- e) Average time of appearance of tumors after transplantation was 25 months.
- f) Thymus was not removed from any patient.

g) Antilymphocytic globulin was given in 10 of 42 patients.

An unusual feature of lymphomas in this series was the frequency with which the brain was involved. Of 22 patients with lymphomas, the brain was involved in 11 and was the only organ involved in eight patients. There is enough evidence at least in animals that acceleration of metastasis is more frequent with azothiaprism and prednisone therapy.¹⁴ This may be true in humans.

Spontaneous cure of cancer

Spontaneous cure of cancer has been a puzzling curiosity for many years. It suggests

Immunological aspects of neoplastic diseases

yet another evidence for the existence of immunological surveillance.

Everson and Cole studied 176 cases of cancer showing spontaneous regression. More than half fell into one of the four categories: a) malignant melanoma, b) neuroblastoma, c) carcinoma of kidney, or d) choriocarcinoma. Lymphomas, leukemias and soft tissue sarcomas are other examples of occasional regression. Though spontaneous regression does not unequivocally prove the presence of tumor specific antigen, it is probably the most logical explanation.

More direct evidence for spontaneous regression comes from histological findings. In breast cancer, a positive correlation exists between favorable prognosis and lymphoid infiltration around the tumor where the tumor is advancing and infiltrating. This lymphoid and plasma cellular reaction suggests specific immunological reaction to some component of tumor growth. Similarly, highest survival rates with gastric carcinomas are associated with lymphoid infiltration in the tumor itself and sinus histiocytosis around regional lymphnodes whether or not lymphnode metastasis is present. It is interesting that these considerations have a higher correlation with survival than adequate surgical removal of tumor.

The remarkably high cure obtained with chemotherapy in Burkitt's lymphoma and choriocarcinoma are attributed to concomitant immunity against strong foreign cell surface antigens. The Epstein-Barr virus related antigen in Burkitt's lymphoma may be responsible for the occasional spontaneous regression seen. Where Burkitt's lymphoma is resistant to chemotherapy, the patients seem to accumulate an IGG layer on the plasma membrane. This globulin apparently renders the cells more immuno-resistant. The 70% cure rate seen in choriocarcinoma, particularly with methotrexate, appears to come from an effective immune response in these patients.

Mechanisms of immunological failure

Precancerous lesions in man can antedate the emergence of frank malignancy by years, ie:

- i) Intestinal polyps preceding colonic carcinoma.
- ii) Solar keratosis and leukoplakia preceding invasive squamous carcinoma.
- iii) Dysplastic squamous epithelium of cervix and bronchus converting into carcinoma cervix and bronchus.
- iv) Hydatiform mole converting to choriocarcinoma.

Immunological surveillance in all these conditions does not always prevent development of tumor. So, what factors are responsible for escape of this immunological surveillance? Postulations are various.^{1,14}

- i) Cancer could be a consequence of an inherited selective defect in immunological surveillance. Leukemia induced by gross virus is seen in 100% of susceptible mice, only 1% of resistant strains after exposure to 87 days in both groups. However, leukemia developed in 25% of resistant strains after 304 days when the immunological surveillance was broken down.¹⁵
- ii) Since neoplasm generally grows from small foci of cells, these may not provide sufficient antigenic stimulus at an early stage of tumor growth to elicit an immunological reaction. Therefore, they sneak through the immunological defense of the host. When the host is sensitized, it may be too late to attack an already established tumor.
- iii) Failure of immunological surveillance may be due to tumor specific antigen inducing a state of immunologic tolerance.
- iv) TSA may be coated by substances such as sialomucin and, therefore, not recognized unless the coating is removed.

Immunotherapy

As the latest modality of approach for treatment of neoplastic diseases, immunization may be prophylactic against a tumor the patient is yet to encounter or therapeutic against one that is already growing.

(1) *Prophylactic immunization.* The ultimate goal of immunotherapy is prophylactic. Experimental models have demonstrated the possibility of such immunization at two levels—the causative agent and the tumor specific antigen of neoplastic cell.¹⁵ The most important target of this futuristic approach to therapy is the oncogenic viruses isolated from human tumor. But two basic problems face a prophylactic vaccine against tumors:

a) Each histologically different neoplasm appears to have a different TSA. So, a new vaccine would be necessary for each neoplasm arising from every organ site, eg, colon, breast, lung, etc.

b) It is unlikely that all human tumors are caused by the same virus. So, a separate vaccine would be needed for each type of virally-induced neoplasm.

In contrast, tumor vaccines based on non-specific immunization might be feasible much sooner. Studies have shown that Bacille Calmette-Guerin (B.C.G.) significantly decreased the development of many spontaneous tumors in several strains of mice.¹⁷ Therefore, nonspecific stimulation of immune response would have a greater advantage over specific vaccine, since, theoretically, it could be effective against all types of human neoplasia. Preliminary evidence suggesting the validity of such a statement comes from the work of Rosenthal and associates who reported a significantly lower incidence of acute leukemia in chil-

dren receiving B.C.G. vaccine compared to control groups.¹⁸

(2) *Therapeutic approach of malignancy* may be:

- a) Nonspecific immunotherapy,
- b) Specific immunotherapy, or
- c) Adoptive transfer of immunity.

(A) *Nonspecific immunotherapy* is an attempt to stimulate the immune system with adjuvants that have no antigenic relationship to the tumor. Several microbiological agents are shown to be capable of general stimulation of the lymphoreticular system. In current clinical trials the most commonly used materials are:^{16,19}

1. Bacillus of Calmette-Guerin (B.C.G.)
2. Methanol extraction residue of B.C.G.
3. Corynebacterium parvum
4. Levamisole
5. Polynucleotides and polysaccharides

In experimental models these agents have inhibited the development of spontaneous tumors, the induction of tumors by chemicals and viruses and the growth of transplantable tumors.^{16,17} Nonspecific immunotherapy seems to promote both cell-mediated and humoral immunity and may be effective in partially reversing the immunosuppression due to tumor and chemotherapy. B.C.G. and corynebacterium parvum are receiving the most extensive clinical trials at the present time.¹⁹ B.C.G. trials are directed against malignant melanoma, acute leukemia and soft tissue sarcomas. In melanoma B.C.G. has produced up to 90% regression²⁰ rate when injected intralesionally. The response to B.C.G. in melanoma occurs primarily in those with disease limited to skin, sub-

Immunological aspects of neoplastic diseases

cutaneous tissue and regional lymphnodes. Patients with metastasis to parenchymal organs such as lung, liver, and brain generally have little response to B.C.G.^{20,25}

Effects of B.C.G. in acute leukemia have mixed results. Mathe²¹ conducted a controlled clinical test in 30 patients with acute lymphatic leukemia who are in complete remission following intensive chemotherapy. Ten of these patients who received no further therapy relapsed within 130 days; the other 20 patients receive irradiated leukemia cells, B.C.G. or both. There was a significant prolongation of survival in the latter group. But, studies carried out by Childrens' Cancer Study Group A showed that the group maintained with drugs had relatively longer durations of remission than either the control group or B.C.G.-treated group. Results of further studies are awaited. Initial trials of B.C.G. in acute myelogenous leukemia are encouraging.²²

Besides intralesional injection, B.C.G. has also been given by aerosol inhalation post-operatively to patients with lung cancer. In addition B.C.G. has been introduced into the pleural space subsequent to lung resection in order to reproduce the beneficial effects of postoperative empyema. But it is too early to evaluate the clinical value of these approaches. Systemic administration of B.C.G. has also been studied²³ as many cancers are already disseminated by the time of diagnosis. In patients with leukemia and melanoma, B.C.G. has also been used either by scarification or the multiple puncture method.²³

Mechanism of action of B.C.G. Why should the injection of B.C.G. cause a tumor to regress? The answer is not entirely clear, but it is known that B.C.G. induces a local sustained granulomatous inflammatory response.^{23,25} As part of this response, various cytotoxic substances are released and all

cells in the immediate vicinity of the tumor, as well as normal cells, may be damaged or destroyed. In addition, macrophages activated into a state of increased phagocytic and metabolic function seem to be able to kill tumor cells.

(B) *Specific immunotherapy* employs substances that are antigenically related to or products of the tumor, such as killed tumor cells from the same patient or from another patient with antigenically similar tumor.

The approach most widely studied is to alter the tumor cells with neuraminidase. This enzyme removes the coating of sialic acid that coats many of the tumor cells, rendering the tumor more immunogenic. Active specific immunotherapy may also include the use of soluble tumor antigen extracts prepared from tumor obtained at surgery or at autopsy. Another approach is to stimulate the patient's lymphocytes with his own tumor cells (or antigen) by cultivating them together in vitro. The lymphocytes immunized in vitro can then be reinfused into the patient, where they may have an increased capacity to interact with tumors.

(C) *The adoptive transfer of immunity* involves transfer of specific immunity from one individual to another. Two sources of material, transfer factor and immune RNA, appear to be capable of transferring immunological information. Both transfer factor and immune RNA can be used to stimulate lymphoid cells in vitro or they may be injected directly into the recipient.

Transfer factor is a cell-free leukocyte extract capable of transferring delayed hypersensitivity.²⁶ It resists digestion by endogenous nucleases, lysosomal hydrolase, deoxyribonuclease and ribonuclease. It is dialysable. Transfer factor is neither antigenic nor an immunoglobulin fragment and has a molecular weight of less than 10,000.

Transfer factor has been used in a wide variety of disorders²⁷ both malignant and non-malignant, commonly where there is an immunodeficient state. The various disorders in which transfer factor is useful are shown in Table V.

TABLE V
CONDITIONS IN WHICH TRANSFER FACTOR IS USED

- Wiskott-Aldrich Syndrome
- Mucocutaneous Candidiasis
- Combined Immunodeficiency Disease
- Coccidioidomycosis
- Verruca Vulgaris
- Dysgammaglobulinemia
- Behcet's Disease
- Aphthous Stomatitis
- Linear Morphea
- Familial Keratoacanthoma
- Partial Di George
- Ataxia Telangiectasia
- Malignant Melanoma
- Osteogenic Sarcoma
- Hypernephroma

(Modified From: "Therapeutic Uses of Transfer Factor" by H. H. Fudenberg et al Hospital Practice January 1974

Immune RNA's^{28,29} exact mechanism in transfer of immunity is unknown. The theoretical advantages in support of adoptive immunotherapy with immune RNA are:

- i) If it is not of itself antigenic even after repeated injections, secondary syndromes or immune elimina-

tions would not be expected to occur.

- ii) There is no danger of inducing a graft versus host reaction in the immunosuppressed host.

The potential sources of immune RNA are:

- i) From lymphocytes of patients who have been cured of tumor or who have undergone spontaneous regression.
- ii) From animals that have been specifically immunized with human tumor.

Immune RNA has been used in renal cell carcinoma with some success. Pilch et al reviewed results of xenogenic RNA immunotherapy in 17 patients with various malignancies.²⁸ One patient showed improvement and had stabilization of disease. The studies with immune RNA²⁹ are still in early stages and further trials are needed to assess the value of this form of immunotherapy. There have been no toxic reactions reported with immune RNA. The major hazard is thought to be potential sensitization to sheep protein and subsequent prophylaxis. Some patients may have slight discoloration of the skin.^{28,29}

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Immunological aspects of neoplastic diseases

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Radhakrishna

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