

## Controversies in Experimental Dermatology

Section Editor: Ralf Paus, Hamburg

# What is the most promising strategy for the treatment of metastasizing melanoma?

Cascinelli N, Heerlyn M, Schneeberger A, Kuwert C, Slominski A, Armstrong C, Belli F, Lukiewicz S, Maurer D, Ansel J, Stingl G, Saida T.  
What is the most promising strategy for the treatment of metastasizing melanoma?

Exp Dermatol 2000; 9: 439–451. © Munksgaard, 2000

N. Cascinelli, M. Heerlyn,  
A. Schneeberger, C. Kuwert,  
A. Slominski, C. Armstrong,  
F. Belli, S. Lukiewicz,  
D. Maurer, J. Ansel, G. Stingl  
and T. Saida

The treatment of patients with metastasizing melanoma, still one of the most deadly diseases in modern medicine, ranks among the greatest challenges that a clinician has to face. Metastatic melanoma also is one of the most profound sources of clinical frustration, since it provides far more ultimately defeating experiences than clinical victories. At the same time, the fascinating biology of melanoma has invited the study of this neuroectodermal tumor as a model system for dissecting many of the key problems of modern oncology, ranging from molecular oncogenesis via the controls of tumor proliferation, apoptosis, invasion, metastasis, and angiogenesis to tumor immunosurveillance and tumor drug resistance. Together with the dire need to develop more effective treatment modalities for improving both life expectancy and quality of life of affected patients, this has made metastatic melanoma a favorite model

for the exploration of innovative strategies for tumor management. Encouragingly, many of these have already generated very promising results in animal models. However, this impressive level of research progress in conquering melanoma in the animal room contrasts rather pitifully with the actual progress made on the ward. This **CONTROVERSIES** feature, therefore, critically and soberly reviews the state of the art of treating metastatic melanoma today (distinguishing between nodal and distant metastases), and sharply defines unresolved or comparatively neglected key problems. In addition, this feature highlights several novel, provocative, hitherto underappreciated, yet potentially promising treatment approaches that deserve systematic exploration. Hopefully, this will offer further inspiration for the design and pursuit of innovative anti-melanoma strategies off-the-beaten-track.

## Viewpoint 1

Melanoma is one of the tumors that most frequently metastasizes to regional lymphnodes and to distant sites. The high degree of malignancy and the poor final outcome correlated with this disease has strongly stimulated in the past decades many studies and clinical trials to help defining the most suitable therapy in these cases. Even though in the area of treatment of nodal metastases important innovations have been introduced in the recent past, for distant metastases, no convincing solution is available at the moment.

### How to treat nodal melanoma metastases today

Surgery remains the only effective option for treating nodal metastases from cutaneous melanoma since chemotherapy and radiotherapy do not achieve the same cure in patients with nodal disease. However the indication for performing an elective lymph node dissection (ELND) or a delayed one (DLND) is contested (1–8).

The supporters of ELND invoke the following arguments: 1) the significant incidence of silent

metastases in stage I/II (AJCC classification); 2) the possibility to select, on the basis of tumor thickness and ulceration, who might benefit from ELND, since patients with intermediate thickness melanoma (1.0 to 4 mm) have an increasing risk of occult regional metastases, but a relatively low risk of distant disease; 3) the low incidence of complications related to these surgical techniques; 4) the results of non-randomized studies that seem to have demonstrated improved survival for patients having ELND.

However several authors don't agree with the necessity to perform an ELND, and raise these points: 1) the absence of conclusive evidence for the hypothesis that melanoma spreads sequentially, first to regional lymph nodes, and later to distant sites after hematogenous dissemination. Instead, there is some evidence that metastatic spread does not always traverse the regional lymph node: the WHO Melanoma Group Register demonstrated only 51% of recurrences present first in the regional lymph nodes, while 22% relapsed first at a distant site, and 31% had simultaneous regional nodal and distant metastases); 2) only a minority of stage I (I-II AJCC) patients submitted to ELND show evidence of nodal involvement at histological examination; 3) post-operative morbidity and negative cosmetic consequences are factors to be considered seriously in this type of surgery; and 4) no conclusive evidence of an improved survival rate after ELND is available. In fact, much of the enthusiasm for ELND is based on retrospective studies that may have been subject to selection bias (1, 2).

However, even if a clear benefit on overall survival has not yet been shown in patients undergoing ELND, a resection of metastatic disease at an early point of the natural course of the disease, while it is still confined to the regional lymphatic basin, definitely is a goal to be strongly pursued in any cancer patient. This could allow, as suggested by some authors (3, 4), the reduction of the risk of spread to distant sites. Furthermore early diagnosis may permit early adjuvant treatment when this may be most active.

Morton and coworkers (9, 10) accordingly have developed a minimally invasive, intraoperative lymphatic mapping technique that should allow more precise pathologic staging before deciding to perform radical dissection. Using this diagnostic procedure, patients with clinically occult nodal disease could be distinguished from those whose lymph nodes are cancer-free. Radical lymphadenectomy is then confined to those in the former group, introducing in this way the concept of selective dissection (in contrast to ELND and DLND of the past). The lymphatic mapping technique uses a blue dye, and subsequently this dye together

with a radioactive agent, to trace the path of lymph as it flows from the primary melanoma to nodes in the regional lymphatic drainage basin. If the melanoma has metastasized, cancer cells are most likely to be found in the lymph node on the lymphatic drainage channel closest to the site of the primary cutaneous melanoma. The "sentinel" node (S.N.) is the first one in the regional basin to be stained as dye enters the lymphatic basin. It can then be removed for immediate pathologic staging before the decision is made to go ahead and perform radical dissection.

It was shown initially in a feline model, and later in humans, that if the S.N. is negative, it's unlikely that other higher nodes contain micrometastatic disease. Studies by Morton (10) showed a very low chance of having skipped metastases in patients with melanoma who were undergoing S.N. biopsy followed by a complete nodal dissection. Accuracy and safety of the technique are now largely documented. The feasibility of the procedure of intraoperative lymphatic mapping is mainly demonstrated comparing the incidence of metastases in sentinel draining nodes with that in secondary non-draining nodes. All the reported experience (11-26) indicates that the S.N. is the lymph node most likely to harbor metastatic melanoma. If the blue-stained or radiolabelled node is negative for metastatic melanoma, then the incidence of metastatic melanoma in the remaining lymph nodes is extremely low.

This approach could reduce the number of unnecessary lymphadenectomies, and helps to apply the procedure to those who are most likely to benefit, introducing the concept of selective dissection as optional alternative to ELND or DLND. Selection of cases and the possibility to perform the procedure in an outpatients setting represent an enormous advantage of the technique in terms of morbidity and cost control. Since many of the ongoing systemic adjuvant trials require nodal involvement as an entrance criterion, use of the sentinel node biopsy followed by selective lymphadenectomy will enable patients to go into trials earlier in the course of disease.

One limitation of the procedure could be seen in the fact that experience is a crucial point in the mastery of this technique. In effect, we are strongly convinced that lack of identification or an incomplete identification of S.N., which could be responsible for the sporadic local relapses referred to in some series (14, 24), probably reflects technical difficulties due to the surgeon's learning curve. The introduction of modalities of identification of S.N. other than blue dye, such as lymphoscintigraphy or probe-guided biopsy, should improve consistently the possibility to identify the S.N. even

through a minimal surgical access (22–25). As a matter of fact, the use of radiotracers for detection of the S.N. (e.g. human serum albumin or sulphur colloid coupled to  $^{99m}\text{Tc}$ ) has strongly improved the original technical procedures.

However, despite all these positive findings, so far we still do not know the exact prognostic significance of early diagnosis of micrometastases in melanoma patients by this technique. Benefit was indirectly deduced in a recent paper from our group (26) reporting the 11-year follow up results of Trial 14 of the WHO Melanoma Programme. According to this analysis, patients with trunk melanoma of 1.5 mm or thicker undergoing elective dissection did not receive any benefit from the procedure. However, the subgroup with clinically occult node metastases discovered at elective dissection showed better survival when compared with patients who had a therapeutic dissection performed at the time of clinical appearance of nodal involvement. Moreover, the results of a recently published multi-institutional study demonstrate the strong prognostic value of S.N. status with respect to disease-free and disease-specific survival (27). This suggests that the extent of metastatic disease inside the nodal basin dissected may play a role in affecting patient survival, and – viewed from this perspective – the excision of nodes at the very onset of metastatic involvement is the goal to be accomplished. On this basis, today, S.N. biopsy must be considered the mandatory modality of diagnosis and treatment of all patients affected with melanomas of 1 mm thickness or more (Table 1). This simple technique has revolutionarized our approach to managing regional nodes in patients

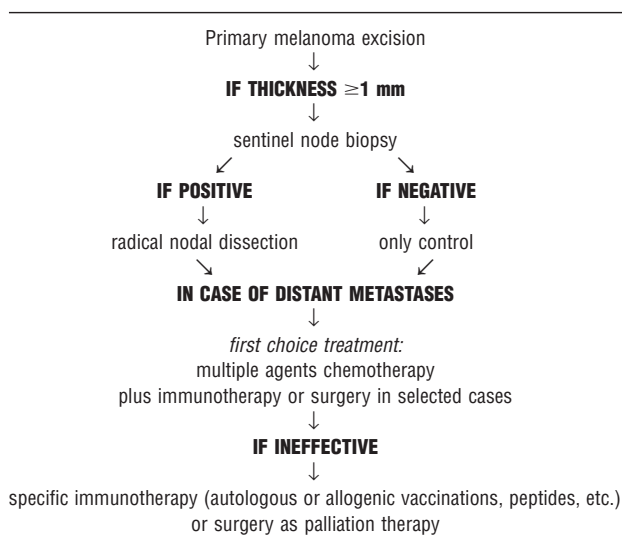
with cutaneous melanoma, finally settling the debate between advocates and opponents of ELND and DLND, limiting the morbidity and expenses of a complete dissection only to those patients with evidence of nodal metastatic disease.

### How to treat distant melanoma metastases melanoma today

There are presently three treatment modalities for diffuse localizations of disease in melanoma: 1) chemotherapy or 2) immunotherapy and 3) surgery that should be considered as alternative approaches in case of inefficacy of previous treatments or that may be applicable in selected cases (Table 1).

- 1) Systemic metastasis from melanoma carries an extremely poor prognosis. Fewer than 5% of melanoma patients with systemic metastases survive five years or more. Standard chemotherapy provides complete tumor response rates of less than 5%, and partial tumor response rates which rarely exceed 25%. The standard agent for melanoma is DTIC, which produces response rates not exceeding 20%.
- 2) Considerable interest has been generated in the last decade by the possibility of immunotherapy for this immunogenic tumour. Many lines of therapy have been tested in the past years with unconvincing results. More recently, gene-modified whole-cell vaccines, newer constructs involving cell membranes on synthetic beads, and purified immunogenetic peptides were evaluated (28–30). To date, response rates for any of the currently available immunotherapeutic modalities do not exceed those of chemotherapy, although some recent studies involving multiple agents, chemotherapy and immunotherapy (biochemotherapy) have suggested that higher complete response rates may be achievable (31, 32).
- 3) Another important aspect to be considered more seriously is that, in selected cases, surgery is now emerging as a valid therapeutic option. As a matter of fact, the surgical approach to melanoma patients with distant metastases is always critical because common clinical evaluation and imaging techniques usually underestimate the tumor load and the effective extent of the disseminated disease. Nevertheless, it has become widely accepted that surgery represents a powerful therapeutic tool for the treatment of metastatic disease in selected melanoma patients. According to many authors, the determinants of survival benefits are successful complete resection of all clinical disease, single versus multiple metastatic site, and anatomic

Table 1. General guidelines for treatment of metastases in cutaneous melanoma



location of disease (33–36). Surgical excision of solitary visceral metastases from melanoma may result in an improved patient survival for up to an overall median of 18 months, especially for patients with intraabdominally localized metastases (34). The metastatic site does not influence outcome, but surgery on a single metastatic sites offers a better life expectancy than on multiple sites, and a radical excision provides better outcome than partial debulking (35, 36). So far, the primary goals of surgical treatment of metastatic melanoma should be the palliation of symptoms, preventing lesions to become symptomatic and, when achievable, the improvement of the patient's life expectancy (reported mean prolongation of life expecting: 7–9 months). Resection, therefore, should be considered for intestinal, isolated pulmonary, intraperitoneal or selected cerebral metastases.

*Natale Cascinelli*

*Filiberto Belli*

National Cancer Institute

I-20133 Milano, Italy

e-mail: cascinnelli@istitutotumori.mi.it

## References

1. Veronesi U et al. *N Engl J Med* 1977; 297: 627–630.
2. Veronesi U et al. *Cancer* 1982; 49: 2420–2430.
3. Balch C M et al. Elective lymph node dissection: Pros and

- Cons. In: Balch C M et al. eds. *Cutaneous melanoma Philadelphia*: Lippincott, 1992: 345–366.
4. Morton D et al. *Ann Surg* 1991; 214: 491–501.
5. McCarthy W H et al. *Surg Gynecol Obstet* 1985; 161: 575–580.
6. Balch C M et al. *Surgery* 1979; 86: 343.
7. Reintgen D S et al. *Ann Surg* 1983; 198: 379.
8. Roses D F et al. *Ann Surg* 1985; 201: 103.
9. Morton D L et al. *Arch Surg* 1992; 127: 392–399.
10. Morton D L et al. *The Melanoma Letter* 1992; 10: 1–4.
11. Cochran A J et al. *World J Surg* 1992; 16: 214–221.
12. Morton D L et al. *Surg Oncol Clin North America* 1992; 1: 247–259.
13. Wong J K et al. *Ann Surg* 1991; 214: 637.
14. Krag D N et al. *Arch Surg* 1995; 130: 654–658.
15. Alex J C, Krag D N. *Surg Oncol* 1993; 2: 137–143.
16. Alex J C et al. *Surg Oncol* 1993; 2: 303–308.
17. Reintgen D et al. *Ann Surg* 1994; 220: 759–767.
18. Uren R F et al. *J Nucl Med* 1993; 34: 1435–1440.
19. Ross M I et al. *Sem Surg Oncol* 1993; 9: 219–223.
20. Morton D L et al. *J Clin Oncol* 1993; 110: 1751–1756.
21. Kroon B B R et al. *Eur J Cancer* 1995; 31A (Suppl. 5): S133.
22. Albertini J J et al. *Ann Surg* 1996; 223: 217–224.
23. Thompson F J et al. *Mel Res* 1995; 5: 255–260.
24. Belli F et al. *Tumori* 1998; 84: 24–28.
25. Joseph E et al. *Ann Surg Oncol* 1998; 5: 119–125.
26. Cascinelli N et al. *Lancet* 1998; 14: 793–796.
27. Gershenwald J E et al. *J Clin Oncol* 1999; 17: 976–983.
28. Pardoll D. *Curr Op Immunol* 1992; 4: 619–623.
29. Arienti F et al. *Hum Gen Ther* 1994; 5: 139.
30. Mulligan R C. *Science* 1993; 260: 926–932.
31. Legha S et al. *Cancer* 1989; 64: 2024–2029.
32. Wadler S, Schwartz E L. *Cancer Res* 1990; 50: 3473–3486.
33. Barth A et al. *J Am Coll Surg* 1995; 181: 193–201.
34. Overett T K and Shiu M H. *Cancer* 1985; 56: 1222–1230.
35. Meyers M L and Balch C M. *Diagnosis and treatment of metastatic melanoma*. In: Balch C M et al. eds. *Cutaneous Melanoma 3rd Edition*, QMP Inc. 1998: Chapter 22: 350.
36. Lejeune F J et al. *Semin Surg Oncol* 1992; 8: 381–391.

## Viewpoint 2

My answer to the question “what is the most promising strategy for the treatment of metastasizing melanoma?” is simple. No treatment is promising at present!

Many regimens of polychemotherapy for advanced melanoma were reported as promising in single-institution phase II trials, but they were almost always followed by disappointing results in larger or randomized studies (1–4). Dacarbazine (DTIC) is still the most reliable drug for advanced melanoma, producing 15–20% overall response rate (5). However, it has little impact on the survival; long-time survivors are exceptionally rare with this drug.

In recent years, two regimens for advanced

melanoma have attracted our attention. One is the Dartmouth regimen, a combination therapy composed of cisplatin (CDDP), DTIC and carmustine (BCNU) along with tamoxifen. The other is called sequential biochemotherapy, in which chemotherapy including CDDP is immediately followed by administration of interleukin-2 (IL-2) and interferon- $\alpha$  (IFN- $\alpha$ ).

### **Effect of the Dartmouth regimen is not confirmed by randomized trials**

The Dartmouth regimen was first introduced by DelPrete et al. in 1984, showing excellent overall (55%) and complete (20%) response rates in 20 pa-

tients with advanced melanoma (6). Following phase II studies employing the Dartmouth regimen confirmed the high response rate (7–9). According to McClay's summing up of 8 phase II trials of the Dartmouth regimen, the overall response rate was 44% (95% confidence interval was 41.7–56.5%) including 14% of complete response in a total of 384 patients (10). McClay et al. emphasized the importance of tamoxifen in the Dartmouth regimen based on their experience that deletion of tamoxifen from the regimen had resulted in a decrease in the response rate from 52% to 10%. They reported that the effects of tamoxifen on melanoma cells did not depend on the presence of estrogen receptors, and suggested synergistic interaction between tamoxifen and CDDP.

In contrast to these excellent results of the Dartmouth regimen, recently reported prospective randomized trials failed to confirm the efficiency of this regimen (11–13). Rusthoven et al. compared the Dartmouth regimen to a regimen containing a placebo instead of tamoxifen. They could not find significant difference in response rate and in survival between the two arms (11). A more recent randomized study has also demonstrated that response rate and survival are not significantly different between the Dartmouth regimen and DTIC alone (13). Which is more reliable, McClay's meta-analysis of phase II studies or the well-designed randomized trials? The randomized trials are surely superior in the level of evidence provided.

### **Significance of sequential biochemotherapy is not determined yet**

Sequential biochemotherapy, in which chemotherapy including CDDP is immediately followed by IL-2 and IFN- $\alpha$ , was introduced in the early 1990s and attracted attention because of its high response rate (14, 15). A variety of regimens of sequential biochemotherapy have been tried, all showing very high overall response rates, mostly more than 40% (16–21). Complete response rates were also relatively high: 10–30%. More importantly, it was reported that 5–10% long-time survivors were obtained. Legha et al. tried "concurrent" biochemotherapy, i.e., simultaneous administration of chemotherapy (CDDP, vinblastine and DTIC) and biotherapy (IL-2/IFN- $\alpha$ ), and obtained a high response comparable to sequential biochemotherapy (22, 23).

Recently, results of randomized trials or sequential biochemotherapy were reported (24–26). Johnston et al. could not find a significant difference in response rate and in survival between the Dartmouth regimen followed by IL-2/IFN- $\alpha$  and the

Dartmouth regimen alone, though low doses of IL-2/IFN- $\alpha$  were administered subcutaneously in their study (24). In a prospective randomized trial conducted by Rosenberg et al. sequential combination of CDDP, DTIC, and tamoxifen with IL-2/IFN- $\alpha$  was not significantly superior to the chemohormonal therapy alone in response rate (44% vs 27%) as well as in median survival time (15.8 vs 10.7 months,  $P=0.052$ ) (25).

A recently reported interim analysis of the randomized trial by the EORTC, comparing two regimens composed of CDDP, DTIC and IFN- $\alpha$  with or without IL-2, response rates (22% vs 28%) did not differ significantly. However, the number of patients without relapse was significantly higher in the IL-2 group, compared to the non-IL-2 group (10/58 patients vs 2/60 patients,  $P=0.028$ ) (26). Final judgement of the significance of sequential biochemotherapy will be determined after completion of the ongoing randomized trials.

If sequential biochemotherapy is significant, its biological mechanisms should be investigated. In the EORTC study, induction of cytotoxic T lymphocytes (CTLs) reactive with melanoma antigens was suggested in patients of the IL-2 group (26). According to Bernengo et al., patients responding to sequential biochemotherapy showed an increase of IL-12 level, indicating activation of macrophages (27). In our study, using the B16 mouse melanoma and the C57BL/6 mouse system, *in vivo* growth of B16-F1 melanoma was significantly inhibited by sequential biochemotherapy composed of CDDP, IL-2 and IFN- $\beta$  (28). Synergistic effects were observed among the 3 agents in this study, and it was suggested that IFN- $\gamma$  production was causally related to the effects.

### **Development of immunotherapy using melanoma antigen peptides and dendritic cells**

Successful cloning of human CTL recognizing autologous melanoma antigens and identification of antigen peptides presented by HLA-class I were a major breakthrough in tumor immunology (29–31). In addition, precise elucidation of the functions of dendritic cells has had great impact (32). Based on these findings, many sophisticated protocols of immunotherapy have been proposed for malignant melanoma. However, only a few trials have shown a definite clinical response.

Rosenberg et al. treated patients with advanced melanoma using IL-2 and g209–2M, a synthetic gp100 peptide (209–217) in which a methionine replaced the threonine at position 2 to increase binding to HLA-A2 (33). The response rate was 42% (13/31 patients), which is significantly higher than

that obtained with IL-2 alone. In the study by Nestle et al., dendritic cells derived from peripheral blood were propagated *ex vivo* and pulsed with cocktails of melanoma antigen peptides or autologous melanoma tissue lysates (34). The dendritic cells were directly injected into lymph nodes of melanoma patients. In this trial, a response rate of 31% (5/16 patients), including complete responders, was obtained. Increased delayed-type hypersensitivity to melanoma antigens was detected in 11 of the 16 patients. In a more recent study by Thurner et al. (35) autologous dendritic cells propagated *ex vivo* and pulsed with MAGE-3A1, a melanoma antigen peptide, were administered to the patients with far advanced melanoma; 3 vaccinations into the skin and then 2 intravenous injections. Regression of individual metastases, including liver metastasis, was observed in 6 of 11 patients. MAGE-3A1-specific CD8<sup>+</sup> CTL precursors expanded significantly in 8 of the 11 patients after the intracutaneous vaccinations but decreased after the intravenous injections (35).

The route of administration of dendritic cells may be a critical factor, since intravenous injection of dendritic cells adopted in most trials showed little effect. Concerning the reported method of immunization, basic research recently reported by Seo et al. deserves to be noted. They applied TRP-2(181–188), a melanoma antigen peptide, on barrier-disrupted mouse skin of which the cornified layer had been removed by tape-stripping, and succeeded in inducing highly reactive CTLs against B16 mouse melanoma (36). In the mice immunized by this method, growth of B16 melanoma was almost completely inhibited.

Although these studies are exciting, there is a major problem in specific immunotherapy: deletion of melanoma antigens and HLA-class I molecules from melanoma cells, which is commonly observed in advanced melanoma. Melanoma cells thus escape from recognition by CTLs. Can we conquer this problem by using cocktails of various antigen peptides, or by transduction of HLA-class I gene?

### Gene therapy using cationic liposomes containing the IFN- $\beta$ gene

Malignant melanoma is one of major targets of gene therapy. Many protocols have been proposed. However, no definitely effective gene therapy has been established yet.

Our group is now investigating the effect of IFN- $\beta$  gene transduction into melanoma cells using multilayered cationic liposomes as a vector. Human melanoma cells transfected with the hu-

man IFN- $\beta$  gene (0.6  $\mu$ g DNA) produced a substantial amount of IFN- $\beta$  protein (up to 67 U/ml) *in vitro*, and proliferation of melanoma cells was significantly inhibited. Human melanoma nodules transplanted to nude mice completely disappeared after 6 times local injection of the liposomes containing IFN- $\beta$  gene (3  $\mu$ g DNA/injection) (37). In contrast, injection of IFN- $\beta$  protein ( $5 \times 10^4$  U/injection) inhibited the growth of melanoma nodules only slightly.

Why is the transduction of IFN- $\beta$  gene much more effective on melanoma cells compared to IFN- $\beta$  protein? Regarding this point, an interesting paper was recently reported by Hanson et al. (38). They showed endogenous expression of IFN gene is related to increased sensitivity to IFN. Chromosome 9p21, where IFN genes are located, is often deleted in melanoma cells. Thus, by transduction of the IFN- $\beta$  gene, melanoma cells may recover sensitivity to IFN- $\beta$ . Because of a variety of inhibitory effects of IFN- $\beta$  such as direct growth inhibition, stimulation of antigen expression, and inhibition of angiogenesis, IFN- $\beta$  gene transduction is expected to work in various manners *in vivo*.

### Conclusions

At present, there is no established treatment of metastatic melanoma. I believe, however, an explosive development of new treatment modalities is just around the corner. Temozolomide (39), an active derivative of DTIC with potentially superior effects, will soon be more widely available, and rational regimens of more effective biochemotherapy may be established in the near future. Moreover, in due course, immunotherapy and gene therapy will surely become powerful tools for the management of metastasizing melanoma. But at least at present, the most reliable strategy to improve the prognosis of this highly malignant neoplasm is accurate detection at the early curable stages (40).

Toshiaki Saida

Dept. of Dermatology  
Shinshu University School of Medicine  
Matsumoto 390-8621, Japan  
e-mail: tosaida@hsp.md.shinshu-u.ac.jp

### References

1. Garbe C. *Melanoma Res* 1993; 3: 291–299.
2. Atkins MB et al. *Cur Opin Oncol* 1997; 9: 205–213.
3. Buzaid A C et al. In: Balch C M et al. (eds), *Cutaneous Melanoma*, 3rd ed, St. Louis, Quality Medical Publishing, 1998: 405–418.
4. Chowdhury S et al. *Cancer Treat Rev* 1999; 25: 259–270.

5. Hill G J et al. *Cancer* 1984; 53: 1299–1305.
6. Del Prete S A et al. *Cancer Treat Rep* 1984; 68: 1403–1405.
7. McClay E F et al. *Int J Cancer* 1992; 50: 553–556.
8. Foshag L J et al. *Proc Am Soc Clin Oncol* 1993; 12: 396.
9. Mastrangelo M J et al. In: DeVita V T et al. (eds), *Principles and Practice of Oncology PPO Updates*, vol. 5, Philadelphia; Lippincott, 1991: 1–11.
10. McClay E F et al. *Semin Oncol* 1996; 23: 744–753.
11. Rusthoven J J et al. *J Clin Oncol* 1996; 14: 2083–2090.
12. Johnston S R et al. *Br J Cancer* 1998; 77: 1280–1286.
13. Chapman P B et al. *J Clin Oncol* 1999; 17: 3745–2751.
14. Richard J M et al. *J Clin Oncol* 1992; 10: 1338–1343.
15. Khayat D et al. *J Clin Oncol* 1993; 11: 2173–2180.
16. Atkins M B et al. *J Clin Oncol* 1994; 12: 1553–1560.
17. Bernengo M G et al. *Melanoma Res* 1996; 6: 257–265.
18. Antoine E C et al. *Cancer J Sci Am* 1997; 3 (suppl): S16–S21.
19. Thompson J A et al. *Cancer J Sci Am* 1997; 3 (suppl): S29–S34.
20. McDermott D F et al. *Proc Am Soc Clin Oncol* 1998; 17: 507.
21. Richard J M et al. *J Clin Oncol* 1999; 17: 651–657.
22. Buzaid A C et al. *Semin Oncol* 1994; 21: 23–28.
23. Legha S S et al. *J Clin Oncol* 1998; 16: 1752–1759.
24. Johnston S R et al. *Br J Cancer* 1998; 77: 1280–1286.
25. Rosenberg S A et al. *J Clin Oncol* 1999; 17: 968–975.
26. Keilholz U et al. *Proc Am Soc Clin Oncol* 1999; 8: 2043.
27. Bernengo M G et al. *Melanoma Res* 2000; 10: 55–65.
28. Kubo H et al. Sequential chemimmunotherapy with cisplatin, IFN- $\beta$  and IL-2 lowers the outgrowth of B16-F1 melanoma in syngeneic mice. *Melanoma Res* 2000; 10: (in press).
29. Van der Bruggen P et al. *Science* 1991; 254: 1643–1647.
30. Boon T et al. *Ann Rev Immunol* 1994; 12: 337–365.
31. Kawakami Y et al. *J Exp Med* 1994; 180: 347–352.
32. Hart D N J. *Blood* 1997; 90: 3245–3287.
33. Rosenberg S A et al. *Nat Med* 1998; 4: 321–327.
34. Nestle F O et al. *Nat Med* 1998; 4: 328–337.
35. Thurner B et al. *J Exp Med* 1999; 190: 1669–1678.
36. Seo N et al. *Proc Natl Acad Sci USA* 2000; 97: 371–376.
37. Kageshita T et al. Growth inhibition of human melanoma tumors in nude mice by intratumoral injection of liposomes containing human IFN- $\beta$  gene (submitted).
38. Flanson C et al. *Melanoma Res* 1999; 9: 451–456.
39. Middleton M R et al. *J Clin Oncol* 2000; 18: 158–166.
40. Saida T. Malignant melanoma on the sole: how to detect the early lesions efficiently. *Pigment Cell Res* 2000; 13: (in press).

## Commentary 1

Normal melanocytes are tightly controlled by keratinocytes. Keratinocytes, the “masters”, dictate when the melanocytes, the “slaves”, can grow and what cell surface molecules are expressed (1, 2). The keratinocytes need cell–cell contact to establish this control, and the adhesion mediator is E-cadherin. Gap junctions between the cells allow intimate exchange of ions and small molecules (3), but we do not yet know which signaling pathways transmit the control from one cell to the other.

Melanoma cells have escaped from keratinocyte control by shutting off expression of E-cadherin and activating N-cadherin (4). They can now leave the epidermis, invade the dermis and closely adhere to and communicate with fibroblasts and endothelial cells. The “run-away” slave has become a powerful master, accepting growth factors from keratinocytes and directing presence and functions of fibroblasts and endothelial cells in its stroma. The melanoma cells dictate the fibroblasts to produce a scaffolding with matrix proteins and to release growth factors they cannot synthesize on their own but which increase their growth, survival and invasive capacity. The symbiosis has been reversed, and the malignant cells are in the driver seat.

We can experimentally reverse the escape of melanoma cells from the epidermis. Melanoma

cells, even the most aggressive metastatic cells, can come again under the control of keratinocytes if we reestablish the expression of E-cadherin by gene transfer (5). The N-cadherin gene is now down regulated and the cells no longer establish gap junctions with fibroblasts (3). The keratinocytes are again in the driver seat: They can adhere to the E-cadherin-expressing melanoma cells and dictate whether these can grow or not (5). Within a few days, all melanoma cell surface molecules associated with growth, invasion and metastasis are shut-off. Important markers are the  $\beta 3$  integrin subunit that allows biologically early melanoma cells to invade into the dermis (6, 7) and the cell–cell adhesion marker Mel-CAM (8). We do not know the mechanisms how keratinocytes can transmit their signals, but these signals are strong enough to force the melanoma cells back into a slave-like position.

Can we translate this observation into therapy of metastatic disease? If we could induce expression of E-cadherin and guide keratinocytes to the melanoma cells ... an impossible task. However, we can trick the melanoma cells by sending them signals that keratinocytes are next to them to take control. We would not kill the malignant cells but prevent further invasion and growth. To achieve this we need to create a “virtual keratinocyte”.

Powerful new molecular technologies should allow us to find new ways and catch the “run-away slave”.

*Meenhard Herlyn*  
Program of Molecular and Cellular Biology  
Wistar Institute  
Philadelphia, PN 19104-4268  
e-mail: HERLYNM@wista.wistar.upenn.edu

## Commentary 2

Progression from a single malignant cell to widespread metastatic disease is accompanied and driven by mutations within the tumor cells offering them a survival advantage (1). These mutations appear by chance, accumulate within the tumor cells, and lead to an enormous phenotypic heterogeneity of the tumor cell pool. Looking at this scenario from an immunological point of view, one must assume that the emerging functional changes will confer certain proportions of the tumor cells with resistance to immune attack. The quality and strength of these immune escape mechanisms correlates directly with the number of tumor cell divisions.

Consequently, the best treatment of stage IV disease would be its prevention by the use of new generation vaccines early during disease progression, e.g. at the stage of a high risk primary tumor. Ideally, the antigenic spectrum of the vaccine should be as broad as possible. Furthermore, to target immune escape variants, one may add alternative anti-tumor modalities such as the use of antiangiogenic substances as well as certain types of gene therapy, including the suicide gene approach and the attempt to correct the expression of growth-modulating genes in cancer cells, either by the blockade of oncogenes or the expression of wild-type tumor suppressor genes.

Currently, the clinical value of the suicide gene approach as well as corrective cancer gene therapy is limited by the inefficiency of the vector systems available to obtain gene expression in every tumor cell throughout the body. Feasible is the genetic modification of tumor cells, antigen presenting cells (APC) and T cells which can then be used for active and/or passive cancer immunotherapy. Though very promising in animal studies (2), the application of autologous and allogeneic tumor cells genetically

## References

1. Valyi-Nagy I T et al. *Lab Invest* 1993; 69: 152–159.
2. Shih I-M et al. *Am J Pathol* 1994; 145: 837–845.
3. Hsu M-Y et al. *J Cell Sci* 2000: in press.
4. Hsu M-Y et al. *Am J Pathol* 2000: in press.
5. Hsu M-Y et al. *Am J Pathol* 1998; 153: 1435–1442.
6. Meier F et al. *Am J Pathol* 2000: in press.
7. Shih I-M et al. *Cancer Res* 1997; 57: 3835–3840.

engineered to express immunostimulatory molecules had only little impact on the clinical course of stage IV melanoma patients (3).

So far, the most intriguing results were obtained when using dendritic cells (DC) loaded with antigens (4, 5) or fused with tumor cells to hybrids (6, 7). This is best exemplified in the case of metastatic renal cell carcinoma in which vaccination with hybrids consisting of autologous tumor cells and allogeneic DC led to complete remissions in 4 out of 17 patients (7). This clinical response was most likely the consequence of an anti-tumor immune response as evidenced by a vaccination-associated increase of the frequency of M<sub>1</sub>-reactive T cells (assessed by intracellular cytokine staining after stimulation with the specific peptic). One approach to increase the efficacy of DC vaccines would be the coadministration of cytokines such as IL-2 to amplify the T cell response or IFN- $\alpha$  to stimulate the activity of the antigen presentation machinery of tumor cells. Alternatively, one could try to recruit cells of the innate immune system to the tumor sites.

However, all these approaches appear to be limited when the immune system is heavily suppressed by the tumor, as is often the case in stage IV disease. Conceivably, this problem may be overcome by the use of a novel strategy that has been developed for leukemias and certain solid tumors: immunosuppressive but nonmyeloablative, low-dose preparative regimens, followed by peripheral blood stem-cell allotransplants (“minitransplants”) to achieve rapid engraftment of donor T cells and to exploit a graft-versus-tumor (GVT) effect in patients with metastases (8, 9). Such transplants have the advantage of low transplant-related mortality with the establishment of full donor lymphoid chimerism which has the potential

to prevent graft-versus-host disease. It will be interesting to see whether this GVT effect can be boosted by immunizing the patients with DC displaying a broad range of melanoma antigens as a result of RNA transfection (10).

*Achim Schneeberger*

*Dieter Maurer*

*Georg Stingl*

Dept. of Dermatology

Division of Immunology and Infectious Diseases

University of Vienna Medical School

A-1090 Vienna, Austria

e-mail: georg.stingl@akh-wien.ac.at

## References

1. Fearon E R, Vogelstein B. *Cell* 1990; 61: 759.
2. Zatloukal K et al. *J Immunol* 1995; 154: 3406.
3. Schreiber S et al. *Hum Gene Ther* 1999; 10: 983.
4. Nestle F O et al. *Nature Med* 1998; 4: 328.
5. Thurner B et al. *J Exp Med* 1999; 190: 1669.
6. Trefzer U et al. *Int J Cancer* 2000; 85: 218.
7. Kugler A et al. *Nature Med* 2000; 6: 332.
8. Khouri I et al. *J Clin Oncol* 1998; 16: 2817.
9. Childs R W et al. *J Clin Oncol* 1999; 17: 2044.
10. Boczkowski A et al. *J Exp Med* 1996; 184: 465.

## Commentary 3

The only curative therapy of malignant melanoma remains the complete excision of the primary tumor. Once distant metastasis has occurred, currently available treatment options are frustrating, both for the patient and the doctor. As Saida and Cascinelli point out, in inoperable metastasizing melanoma, the standard of treatment still is the monochemotherapy with dacarbazine (DTIC), with response rates between 12–20% (1). After so many years and numerous trials of trying other chemotherapeutics and their combinations to increase response rates, this is a deeply discouraging fact. Recently published data show no significant differences in the response rates after immunochemotherapy, but an effect of interleukin 2 in terms of the so-called “long time survival” (2). This, of course is a big step forward in the treatment of advanced melanoma, yet surely not enough to be happy with.

There is little to be added to the excellent critical reviews of current immuno- and gene therapy strategies provided by the viewpoint authors. However, one key question that deserves greater attention is: What is the difference between the so-called responder and a non-responder, and what can we do to enhance these discouraging response rates? In my view, a critically important issue is to develop better predictive markers for the tumor response to therapy than are currently available.

The control of melanoma growth and melanoma metastasis is the net result of very complex interactions between the individual and the tumor (3). These interactions differ from patient to patient, which makes it currently exceedingly difficult to

predict how the tumor and the patient will react to chemotherapy.

For example, the study of Cree et al., who performed an *ex vivo*, ATP-based chemosensitivity assay, showed a great heterogeneity of chemosensitivity, with some tumors not reacting to any of the tested substances (4). This heterogeneity *ex vivo* is in line with the clinical experience and the known heterogeneity concerning oncogenetic and other molecular changes in melanoma (5). The most active single cytotoxic agents in these *ex vivo*-assays were cisplatin, treosulfan, paclitaxel, vinblastine, gemcitabine and mitoxantrone. The most active combinations of drugs were gemcitabine + treosulfan, cisplatin + paclitaxel and vinblastine + paclitaxel (4). Due to these observations there are ongoing trials with these single agents or the aforementioned combinations.

To enhance melanoma sensitivity to chemotherapy, the apoptosis control machinery, including known BCL-2 mutations, may be another important target of therapy. In an SCID mouse model, BCL-2 oligoantisense therapy dramatically enhanced tumor sensitivity to DTIC (6), which has already encouraged the start of a clinical trial with this combination (7).

Another intriguing strategy for the treatment of metastasizing melanoma are oncolytic viruses. Two independent studies, one with Newcastle disease virus (8), another with vaccinia melanoma oncolysate vaccine (9), have shown most encouraging effects in the adjuvant setting. Why not trying it for metastasizing melanoma, as well?

Personally, I am particularly fascinated by an-

other potentially very interesting substance in the treatment of melanoma: azelaic acid. This C9 dicarboxyl acid was initially used in 1980 as an alternative in the local treatment of primary cutaneous malignant melanoma (10). The good clinical response encouraged Nazzaro-Porro et al. to try it in advanced melanoma and to check its antitumoral efficiency (11, 12). Subsequently, many antitumor-activities of azelaic acid were demonstrated. For example, azelaic acid significantly inhibits the growth of murine and human melanoma cell lines and of other tumor lines *in vitro* (15, 16), and reportedly has a significant chemo-sensitizing effect on melanoma cell lines (17). Intriguingly, this substance has no known LD 50, no mutagenic or teratogenic effects, and has even been investigated as a possible substance for parenteral nutrition (13, 14). As a consequence, we are now starting a clinical trial with azelaic acid in combination with temozolomide (selected because of its excellent bioavailability, including the capability to pass the blood–brain barrier, and the possibility of oral administration (18)).

Thus, although the treatment of metastasizing melanoma is still quite frustrating, there are well-defined and theoretically promising strategies that deserve to be systematically explored.

Christoph Kuwert  
Dept. of Dermatology  
University Hospital Eppendorf  
University of Hamburg  
D-20246 Hamburg, Germany  
e-mail: kuwert@uke.uni-hamburg.de

## Commentary 4

The metastatic phase of malignant melanoma poses a serious management problem, since it is generally resistant to the currently available methods of therapy (1). A promising strategy could be represented by immunotherapy that would activate cellular and humoral responses against the tumor (1–4). However, optimization of the immunotherapeutic approaches requires in-depth understanding of the biochemistry of melanoma cells. In this context, the capability of melanoma cells to synthesize melanin and to express the enzymatic and structural proteins regulating the process – an intrinsic characteristic of cells of melanocytic origin (4, 5) – is an indispensable factor in determining the outcome of immunotherapy (6).

## References

1. Luce J K et al. *Cancer Chemoth Rep* 1970; 54: 119–124.
2. Keilholz U et al. *Cancer J Sci Am* 2000; 6 Suppl I: 99–103.
3. Fountain J W. In: Balch C M et al. (eds), *Cutaneous Melanoma*, 3rd ed, St Louis: Quality Medical Publishing, 1998: 475–492.
4. Cree I A et al. *Anti Cancer Drugs* 1999; 10: 437–444.
5. Fidler I J. In: Balch C M et al. (eds), *Cutaneous Melanoma*, 3rd ed, St Louis: Quality Medical Publishing, 1998: 493–516.
6. Jansen B et al. *Nature Medicine* 1998; 4: 232–234.
7. Jansen B et al. Phase I–II Study with Dacarbazine and BCL-2 Antisense Oligonucleotide G3139 (GENTA) as a Chemosensitizer in Patients with Advanced Malignant Melanoma. 35th Annual ASCO Meeting Abstract, 1999.
8. Batliwalla F M et al. *Molecular Medicine* 1998; 4: 783–794.
9. Wallack M K et al. *Ann Surg* 1997; 226: 198–206.
10. Nazzaro-Porro M et al. *Lancet* 1980; 1: 1109–1111.
11. Nazzaro-Porro M et al. *Clin Exper Dermatol* 1996; 21: 320–324.
12. Rodriguez P et al. *Int J Dermatol* 1993; 32: 363–364.
13. Passi S. *Contemp Pharmacother* 1993; 4: 441–447.
14. Tacchino R M et al. *J Parent Ent Nut* 1990; 14: 169–172.
15. Picardo M et al. *Biochem Pharm* 1985; 34: 1653–1665.
16. Robins E J et al. *J Invest Dermatol* 1985; 85: 216–221.
17. Rodriguez-Vincente J et al. *Cancer* 1998; 82: 495–502.
18. Newlands E S et al. *Cancer Treat Rev* 1997; 23: 35–61.

The biosynthesis of melanin consists of a series of tightly coupled reactions (4, 5). Throughout this, several intermediates are generated that can display cytotoxic, genotoxic or mutagenic activities if diffusing into the cytoplasm or nucleus (4–8). Nevertheless, in normal melanocytes, the process of melanin synthesis is highly controlled, since it takes place within the boundaries of specialized membrane bound organelles, the melanosomes (9). Ongoing melanogenesis and/or melanin itself also place secondary demands on the metabolic state of the host cell (6, 7). Thus, besides the oxygen requirements imposed by active melanogenesis, which can lead to intracellular hypoxia, melanin can act as scavenger of free radicals, metal cations and many

chemicals, including cellular toxins. Finally, intermediate products of melanogenesis can suppress local and possibly systemic immune responses (6).

### Does melanogenesis affect melanoma progression and therapy?

Therefore, we have proposed that uncontrolled melanogenesis may have a role, perhaps critical, in the progression of melanotic melanoma (6). The associated oxidative environment and the abnormal presence of genotoxic and mutagenic intermediates would mediate this activity. Such factors could destabilize tumor cells and their microenvironment, and may contribute to the generation of malignant phenotype heterogeneity (6). The immunosuppressive action of melanin precursors would promote tumor aggressiveness by allowing tumor escape from a weak host immune response (6).

In addition to its effects on the tumor cells, the tumor responses to radio-, chemo- and phototherapy may also be limited by the relative intracellular oxygen shortage that is produced by active melanogenesis, together with the free radical-scavenging properties of melanin (4, 10). Evidently immunotherapy protocols aimed at the eradication of melanoma cells must also be severely influenced by the immunosuppressive effects of melanogenesis.

### Inhibition of melanogenesis is a viable adjuvant strategy in therapy of melanotic melanoma

Since immunotherapy may be hampered by the immunosuppressive action of ongoing melanogenesis in melanoma cells, we have proposed to inhibit or suppress melanogenesis as an important adjuvant treatment strategy in the management of metastasizing melanoma (6). This strategy for enhancing tumor immunogenicity, together with preventing tumor strategies for evading immunosurveillance, is likely to generate beneficial and synergistic effects, when used in conjunction with immunomodulatory, vaccination and gene therapy protocols (1–4).

The proposed strategy of suppression of melanogenesis could also enhance melanoma cell radiosensitivity by decreasing the intracellular concentration of the radioprotector melanin (10). Moreover, the increased availability of oxygen, which is otherwise consumed by melanogenesis (7), may further augment the response to radiotherapy (10). Thus, inhibition of melanogenesis may improve the outcome of radiotherapy and allow the use of low LET radiation. Additional beneficial effects of melanogenesis inhibition could include increased sensitivity to chemo- and phototherapy.

There are multiple potential targets for mel-

anogenesis attenuation. For example, inhibition of tyrosinase synthesis, processing, activation, or actual enzymatic activity (4, 5); inhibition of the postdopa oxidase steps (11); use of melatonin receptors agonists (12) or of MC 1 receptor antagonists (13); or stringent dietary restriction of L-tyrosine and L-phenylalanine (14). The use of several inhibitors simultaneously could allow the administration of lower doses of individual agents to decrease drug toxicity. Additive melanogenesis-inhibiting therapies are also likely to displace the donor-acceptor equilibrium towards an excess of electron acceptors; such a shift should favor radiosensitization and possibly, the response to chemotherapy.

### Conclusion

Inhibition of melanogenesis represents an interesting novel approach (6) that deserves to be explored as an adjuvant therapeutic modality to improve, in particular, the outcome of immuno- and radiotherapy in metastasizing melanoma, and to reduce the probability of melanoma progression.

*Andrzej Slominski*

Department of Pathology  
University of Tennessee  
Memphis, TN 38163, USA  
e-mail: aslominski@utmem.edu

*Stanislaw Lukiewicz*

Laboratory of Radiospectroscopy of Cancer  
Institute of Molecular Biology  
Jagiellonian University  
Krakow, Poland

### References.

1. DeVita V T et al. eds. Cancer. Principles and Practise of Oncology. Philadelphia: Lippincott-Raven Publishers, 1997.
2. Sakai C et al. *Melanoma Res* 1997; 7: 83–95.
3. Catelli C et al. *J Cell Physiol* 2000; 182: 323–331.
4. Hori Y et al. eds. *Melanogenesis and Malignant melanoma. Biochemistry, Cell Biology, Molecular Biology, Pathophysiology, Diagnosis and Treatment*. New York: Elsevier, 1996.
5. Protá G, ed. *Melanins and Melanogenesis*. New York: Academic Press, 1992.
6. Slominski A et al. *Anticancer Res* 1998; 18: 3709–3716.
7. Slominski A et al. *J Theor Biol* 1993; 164: 103–120.
8. Miranda M et al. *Mutagenesis* 1997; 12: 233–236.
9. Moellmann G et al. *Pigment Cell Res* 1998; Suppl 1: 79–871.
10. Lukiewicz S et al. In: Seiji M, ed. *Pigment Cell. Phenotypic Expression in Pigment Cells*. Tokyo: University of Tokyo, 1981: 647–653.
11. Pawelek J. *Pigment Cell Res* 1991; 4: 53–62.
12. Slominski A, Pruski D. *Exp Cell Res* 1993; 206: 189–294.
13. Siegrist W, Eberle A N. *Trends Endocrinol Metab* 1995; 6: 115–120.
14. Demopoulos H B. *Cancer* 1966; 16: 657–664.

## Commentary 5

The search for effective treatments for human diseases rarely progresses in a linear fashion. The history of therapeutic innovations for cancers such as metastatic melanoma demonstrates how medical science, politics, personalities, and the shared desire to save people from dying from a vast group of neoplastic diseases have often created climates that favor particular forms of therapy while ignoring “conflicting” approaches. A positive climate certainly facilitates the funding, peer-reviewed publications, and public support that allow an anti-cancer approach to be studied and tested. But fortunately, our system of modern experimental medicine eventually forces an unbiased appraisal of even the most popular therapy and ensures that it will be adopted or abandoned based on its actual merits to cancer patients. Conversely, cancer therapy approaches that are widely considered to be of little value can become accepted by scientists and clinicians if careful and ethical preclinical and clinical studies support their efficacy in a reproducible manner.

The use of viruses to treat human cancer falls into the general category of an anticancer approach that is widely considered to be of little value in the USA’s greater than 30 year old “war against cancer.” In contrast, earlier in the 20th century a large number of viruses were studied in human trials based on their oncolytic activities and stimulation of protective immune responses. As our understanding of the intricate workings of the immune system evolved, however, interest in this form of cancer immunotherapy has faded. What has developed is the well-established field of tumor immunology with its clinical arm of tumor immunotherapeutics that cover an incredible breadth and scope of basic and clinical studies designed to better understand and then harness the host immune system to resist early or established neoplasms. The identification and use of potent immunomodulating cytokines, of tumor specific peptide vaccines, and of effector cell populations including subsets of T cells and professional antigen presenting cells as anti-tumor agents have led to significant advances in our understanding of the host response against particular types of neoplastic diseases.

In particular, the study of the immunobiology of metastatic melanoma has contributed significantly to the understanding of tumor immune responses and has made this neoplasm an important target for immunotherapeutic trials. Additionally, the incorporation of gene therapy within tumor

immunotherapy approaches has gained wide acceptance and stimulated great hope for improved treatment options for cancer patients. However, while often promising and certainly worth pursuing, the vast majority of “mainstream” tumor immunotherapy clinical studies have not yet shown meaningful clinical benefits and some incur significant risk and morbidity.

Thus, as scientists and clinicians have continued to explore the use of “alternative” immunotherapeutic approaches to cancer treatment, a handful of researchers are reviving the idea that particular viruses may have useful anti-tumor activities that can be exploited against human disease. Our research group became interested in the use of post-surgical vaccination with Newcastle disease virus (NDV) oncolysate in the adjuvant treatment of metastatic melanoma based on the enhanced survival of a cohort of stage III melanoma patients with palpable lymph nodes treated in a phase II trial that began in 1975 at our institution. The oncolysate was prepared from allogeneic or autologous melanoma cells grown in culture and lysed by the NDV strain 73T developed by Dr William Cassel, a pioneer in NDV therapy.

The 83 melanoma patients enrolled in this study were reported to have a 10 year survival of greater than 60% (1). This result can be compared to the 6–15% 10-year survival of comparable historical patient populations with palpable lymph node disease and the 33% survival rate at 10 years observed in historical studies in which patients with occult lymph node disease were included. We have recently reported that the 15-year survival of these patients remains at 55% (2). Additionally, we evaluated the immunological effects of long term NDV oncolysate vaccination by carrying out a comprehensive analysis of the peripheral blood T cell repertoire in these patients. We detected a striking oligoclonality in the CD8+ T cell population in peripheral blood and clonal expansions in the CD8+ CD57+ subset, which has been previously associated with improved outcome in patients undergoing tumor immunotherapy (2, 3).

For a number of years, other small groups of researchers around the USA and Europe also have continued or initiated studies of the use of other strains of NDV for the treatment of a wide range of solid tumors including malignant melanoma (4). Several phase I and II trials as well as a phase III trial with NDV oncolysate vaccination are currently in progress (4). Together, the results of the clinical trials with NDV oncolysate are no less

promising than the results of the vast majority of cancer immunotherapeutic studies with other agents.

The mechanisms by which NDV oncolysate therapy may mediate effective anti-tumor host immunologic responses are unclear. Several groups of scientists have demonstrated that NDV may function in part through the host activation of cytokines with anti-tumor activities, through changes in the tumor cell membranes, or even through direct lytic effects (5–8). We have also initiated pre-clinical mechanistic studies with a syngeneic murine melanoma system with the aim of advancing our understanding of the specific immunologic mechanisms by which NDV oncolysate may function against metastatic melanoma (9).

It appears likely that this “non-specific” mixture of virus and tumor cell membranes may function to augment the same immune responses that are also stimulated by other widely accepted immunotherapeutic approaches now being tested in clinical trials. It is possible that therapy with NDV oncolysate vaccination may be particularly successful against certain neoplasms or in combination with other treatment modalities because it is “non-specific” and facilitates enhanced presentation of multiple tumor antigens against which an effective host response can thus be generated.

We are hopeful that continued scientifically rig-

orous and ethical studies to investigate the role of NDV oncolysate in the treatment of malignant melanoma will ultimately be evaluated on the merit of the data generated and not on the perception that this approach is of no real value. While the use of viral approaches in the treatment of neoplastic disease has not followed a direct path, viruses such as Newcastle disease virus may yet prove to be valuable agents in our search for effective anti-melanoma therapies.

*Cheryl Armstrong*

*John Ansel*

Dept. of Dermatology

Emory University

Atlanta, GA 30322, USA

e-mail: carms01@emory.edu

## References

1. Cassel W A, Murray D R. *Med Oncol Tumor Pharmacother* 1992; 9: 169–171.
2. Batliwalla F M et al. *Mol Med* 1998; 4: 783–794.
3. Elliott G T et al. *Semin Surg Oncol* 1993; 9: 264–272.
4. Nelson N J. *J Natl Cancer Inst* 1999; 91: 1708–1710.
5. Lorence R M et al. *J Natl Cancer Inst* 1988; 80: 1305–1312.
6. Lorence R M et al. *J Natl Cancer Inst* 1994; 86: 1228–1233.
7. Sinkovics J G. *Int Rev Immunol* 1991; 7: 259–287.
8. Schirrmacher V et al. *Int J Oncol* 1999; 14: 205–215.
9. Bonaccorsi P et al. *J Invest Dermatol* 2000; 114: 798 (abstract 281).