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ORIGINAL PAPER

# Biphasic effect of a primary tumor on the growth of secondary tumor implants

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**Abstract**
*Background* The phenomenon of hormesis is characterized by a biphasic dose–response, exhibiting opposite effects in the low- and high-dose zones. In this study, we explored the possibility that the hormesis concept may describe the interactions between two tumors implanted in a single mouse, such that the resulting tumors are of different sizes.
*Materials and methods* We used two murine tumors of spontaneous origin and undetectable immunogenicity growing in BALB/c mice. A measure of cell proliferation was obtained by immunostaining for Ki-67 protein and by using the [$^3$H] thymidine uptake assay. For serum fractionation, we utilized dialysis and chromatography on Sephadex G-15.
*Results* The larger primary tumor induced inhibitory or stimulatory effects on the growth of the smaller secondary one, depending on the ratio between the mass of the larger tumor relative to that of the smaller one, with high ratios rendering inhibition and low ratios inducing stimulation of the secondary tumor.
*Conclusion* Since metastases can be considered as natural secondary tumor implants in a tumor-bearing host and that they constitute the main problem in cancer pathology, the use of the concept of hormesis to describe those biphasic effects might have significant clinical implications. In effect, if the tumor-bearing host were placed in the inhibitory window, tumor extirpation could enhance the growth of distant metastases and, reciprocally, if placed in the stimulatory window, tumor extirpation would result not only in a reduction or elimination of primary tumor load but also in a slower growth or inhibition of metastases.

**Keywords** Murine tumors · Hormesis · Biphasic dose–response · Secondary tumor implants · Metastases

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## Introduction

The phenomenon of hormesis is characterized by a non-monotonic dose–response that is biphasic, exhibiting opposite effects at low and high doses (Thong and Maibath 2007). Although biomedical sciences have been dominated during the past century by the assumed sigmoidal nature of dose–responses, evidence accumulated over the years suggests that the hormetic dose–response model may be more common and fundamental than previously thought. This evidence was stressed over the past decade as a consequence of more importance being given to low-dose effects and the use of more powerful tools aimed to detect those effects.

In effect, hormetic dose–responses have been reported for many agents designed for the treatment of numerous pathological conditions such as anxiety, memory disorders, seizure, stroke, dermatological, ocular and cardiac diseases, osteoporosis, cancer, etc. (Calabrese 2008a, b; Eichler et al. 2002; Gao et al. 2002; Honar et al. 2004; Wise and Lichtman 2007).

Regarding cancer research, substantial evidence shows that low doses of different anti-tumor agents commonly stimulate the in vitro proliferation of many different types of tumor cells, while high doses inhibit their proliferation or kill them. This is consistent with the hormetic dose–response relationship (Calabrese 2007). Similarly, agents that affect capillary development, and in consequence considered potentially significant in the treatment of neoplastic diseases that depend on angiogenesis, also exhibit a hormetic dose–response, with high doses inhibiting and low doses enhancing the in vitro proliferation of microvascular endothelial cells (Folkman 2007).

In the same way, the immunostimulatory theory, proposed by Prehn many years ago, states that the immune reaction may be either stimulatory or inhibitory to tumor growth depending on the local ratio of immune reactants to tumor cells, with high ratios rendering tumor inhibition, intermediate and very low rendering null effects and low ratios (between intermediate and very low ones) rendering tumor stimulation (Prehn 1972, 2006, 2007). This theory, which is an application of the hormesis concept for immunological reactions, has been supported by numerous data from his and other laboratories (Chiarella et al. 2008a, b; Norbury 1977; Prehn 1976; Shearer and Parker 1978).

Implications of having a stimulatory zone may be clinically significant. In effect, chemotherapeutic, anti-angiogenic and immunological agents designed to kill tumor cells or suppress their proliferation in patients may eventually enhance tumor growth when the concentration of the anti-tumor agent is low (i.e., stimulatory) or reaches a low value in the body some days after treatment is initiated. This could be an important problem for agents with a long half life.

In this study, to know if the hormesis concept can be extended to other fields of cancer biology, we studied the effect of a primary tumor on the growth of secondary tumor implants. This phenomenon has received little attention despite the fact that it has been detected in association with human cancers (Beecken et al. 2009; Lange et al. 1980; Southam 1968; Sugarbaker et al. 1977) and despite its possible relevance to predicting the fate of metastatic foci in tumor-bearing hosts on the basis that metastasis can appropriately be considered as natural secondary tumor implants in a tumor-bearing host (Beecken et al. 2009). Further, the relatively scanty number of papers addressing this phenomenon has rendered controversial results, since inhibitory and stimulatory effects have been reported, depending on the tumor models utilized (McAllister et al. 2008; O'Reilly et al. 1994; Ruggiero et al. 1985).

We have used herein, two murine tumors of spontaneous origin and non-detectable immunogenicity because we wished to explore the phenomenon without confusion from the immune reaction and because murine spontaneous tumors could presumably best be compared with human tumors. We are going to demonstrate that a primary tumor can exert both inhibitory and stimulatory effects on the growth of secondary implants of the same tumor depending on the relative masses of both tumors.

## Materials and methods

### Animals

BALB/c mice, 3–5 months old were used throughout. They were raised in our own colony and maintained on Cooperation pellets (San Nicolás, Buenos Aires, Argentina) and water ad libitum. The care of animals was according to the policies of Academia Nacional de Medicina of Buenos Aires, Argentina (NIH Guide and Use of Laboratory Animals).

### Tumors

LB: It is a non-immunogenic T-lymphoid leukemia-lymphoma that arose spontaneously in a 6-month-old BALB/c male. It was maintained by subcutaneous (s.c.) serial passages in syngeneic mice. A more detailed description of this tumor is given elsewhere (Ruggiero et al. 1985; Zahalka et al. 1993).

CEI: It is a non-immunogenic undifferentiated epidermoid carcinoma, which arose spontaneously in a 12-month-old BALB/c female. It was maintained by syngeneic s.c. serial passages. More details of this tumor are given elsewhere (Meiss et al. 1986).

Tumor volume was expressed according to the formula of Attia and Weiss: volume = 0.4 ($ab^2$) where $a$ and $b$ represent the larger and the smaller diameters, respectively (Meiss et al. 1986). Male mice were used in the experiments for LB tumor and female mice were utilized in the experiments for CEI tumor.

### Histological studies

Skin with or without macroscopic tumor was removed and fixed in 15% formaldehyde, 5% acetic acid and 80% methanol. The tumor was sliced along the largest diameter and embedded with the overlying skin. Serial sections (3–5 μm) were obtained, stained with hematoxylin and eosin and studied.

Mitotic number was evaluated per high power field (HPF) at ×1,000 magnification in well-preserved areas (non-necrotic) with similar cell densities, while taking into account mainly metaphase and anaphase. A measure of cell proliferation was obtained by immunostaining for Ki-67 (Goat polyclonal M19; Santa Cruz), a nuclear protein that is expressed in proliferating cells in the G1, S, G2 and M phases and absent in the G0 phase. Staining was developed under microscope with diaminobenzidine peroxidase

substrate. The number of cells expressing Ki-67 was evaluated with HPF at ×400. In each case, 50 fields (HPF) were counted.

Medium

The medium used was RPMI-1640 (Gibco, Grand Island, NY, USA), with penicillin G sodium (10 μg/ml), streptomycin sulfate (25 μg/ml) and amphotericin B as fungizone (25 μg/ml). The medium was supplemented with 5% fetal calf serum.

Serum

Normal and tumor-bearing mice were bled and the blood was kept at room temperature for 1 h for clotting. Serum obtained after centrifugation was stored at −20°C until use. For [$^3$H] thymidine uptake assays, serum was decomplemented at 56°C for 30 min.

[$^3$H] Thymidine uptake assay

Proliferation of tumor cells in 0.1 ml of medium was determined in 96-well microtiter plates (NUNC, Denmark) in the presence of several twofold dilutions of serum from normal or tumor-bearing mice. Immediately afterward, the cultures were pulsed with [$^3$H] thymidine (Dupont, NEN Research Products, Boston, MA, USA) at a final concentration of 1 μCi/ml, the mixture was incubated at 37°C for 18–24 h in a 5% carbon dioxide humidified atmosphere and harvested with an automated cell harvester. The radioactivity incorporated into the cells was counted in a liquid scintillation beta counter (Beckman). The assays were carried out in quadruplicate or sextuplicate.

Cell-mediated cytotoxicity assay

This test was carried out as previously described (Franco et al. 1996). Briefly, 0.1 ml of $^{51}$Cr-labeled tumor cells was incubated with the same volume of different spleen cell suspension at an effector ratio of 100:1, for 4 h at 37°C in a 5% carbon dioxide humidified atmosphere. Afterward, cells were centrifuged and radioactivity in the supernatant was measured in a gamma counter (Beckman). The percentage of specific lysis was calculated as [(experimental c.p.m. − normal c.p.m.)/(maximal c.p.m. − normal c.p.m.)] × 100.

Serum fractionation

*Dialysis*

Serum from normal and tumor-bearing mice was subjected to dialysis (12,500 molecular weight cut-off).

*Chromatography on Sephadex G-15*

The dialyzable fraction of serum was concentrated by lyophilization, resuspended in 0.5 ml of water and applied to 66 × 0.7 cm chromatographic column of Sephadex G-15; elution was performed with water with a 0.44 ml min$^{-1}$ flow rate. Each fraction obtained was assayed on in vitro proliferation of tumor cells using the [$^3$H] thymidine uptake assay.

Statistical analysis

Values were expressed as mean ± standard error (SE). The Student's *t* test was used and differences were considered significant when $p \leq 0.05$.

## Results

Kinetics of a primary LB tumor

The growth of LB tumor initiated with an s.c. inoculum of $10^6$ LB cells is plotted in Fig. 1. The tumor becomes initially detectable on day 3 and then its size increases progressively killing the host on, approximately, day 22.

Biphasic effect of a primary LB tumor on the growth of secondary LB tumor implants

A total of 42 mice received an s.c. implant of $1 \times 10^6$ LB tumor cells in the right flank on day 0 (primary tumor) and on days 0, 1, 2, 3, 4, 6 and 9, when the primary tumor volume was 0, 0, 0, 10, 50, 250 and 700 mm$^3$, respectively. They received a secondary s.c. implant of $1 \times 10^5$ LB cells in the left flank ($n = 6$ mice per group). Six mice that only received the s.c. implant of $1 \times 10^5$ LB cells in the left

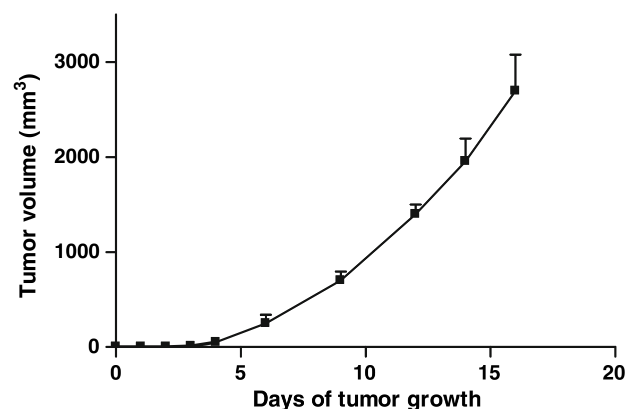


**Fig. 1** Growth of s.c. LB tumor initiated with an inoculum of $1 \times 10^6$ LB cells in the right flank. Each point represents the mean ± SE of 12 mice

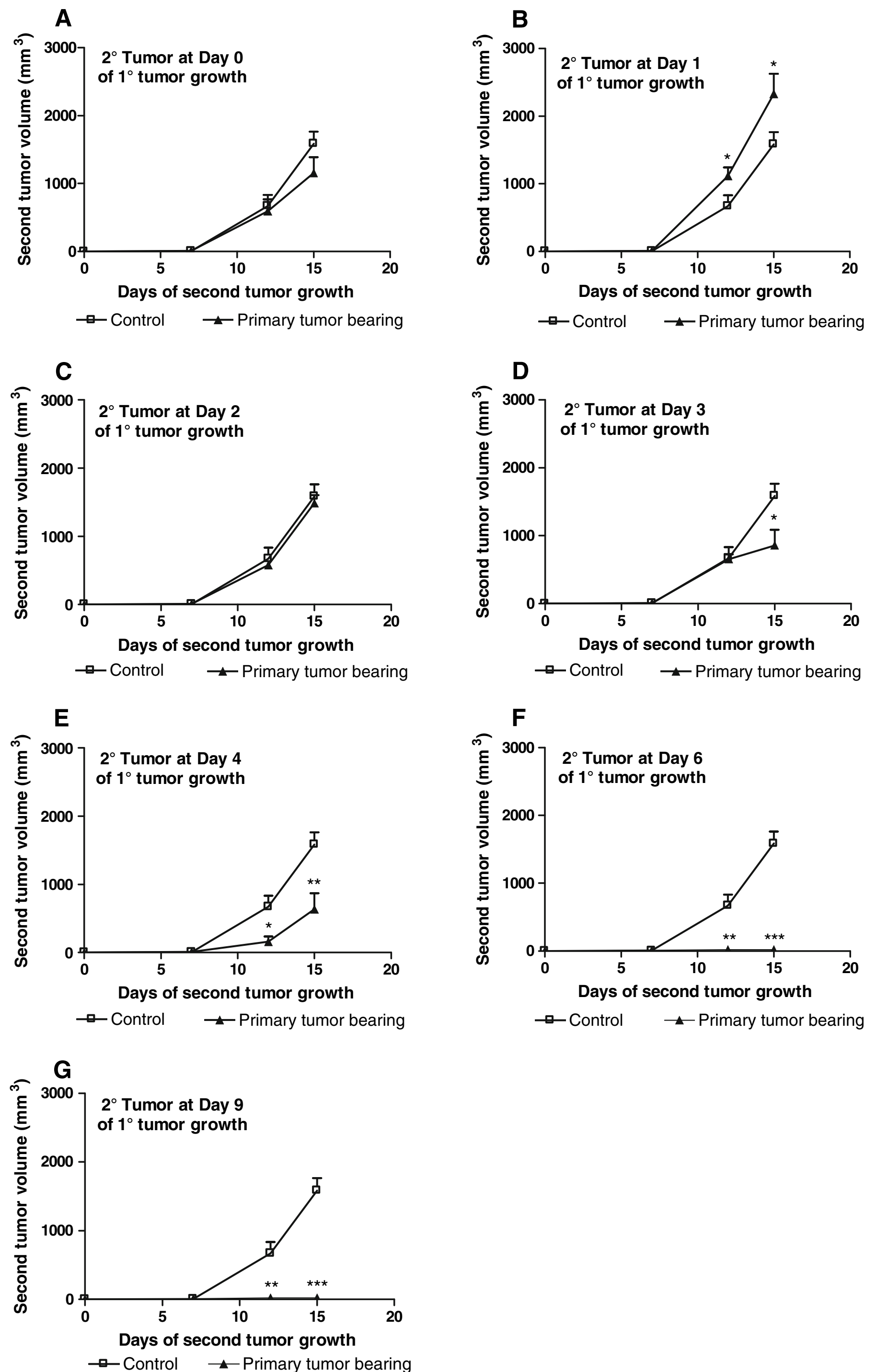


**Fig. 2** Growth of a secondary LB tumor implant in primary LB tumor-bearing mice. The primary LB tumor was initiated with $1 \times 10^6$ tumor cells inoculated in the right flank on day 0. A secondary tumor was implanted ($1 \times 10^5$ LB tumor cells) in the left flank on days 0 (**a**), 1 (**b**), 2 (**c**), 3 (**d**), 4 (**e**), 6 (**f**) or 9 (**g**) of primary tumor growth ($n = 6$ mice per group). Controls were six mice receiving only the inoculum of $1 \times 10^5$ LB cells in the left flank. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

flank (that is, not bearing the primary tumor) served as controls.

As shown in Fig. 2, the primary tumor produced different effects on the second tumor growth, depending on the day on which the second implant was carried out. In effect, when a second tumor was implanted on days 0 or 2, no difference as compared with the control was observed. When the second tumor was implanted on day 1, a significant enhancement was detected. Finally, when the second tumor was implanted on day 3 onwards, a significant inhibition was observed. The most profound inhibition was detected on days 6 and 9, when the primary tumor was the largest at the time of the secondary tumor implant.

Although on days 0, 1 and 2 no primary tumor mass was macroscopically detected, the mass of the primary tumor on the day when the second tumor began to be perceptible

(day 7 of second tumor growth, tumor volume 11 $mm^3$) was different in the cases in which the second tumor was implanted on day 0 (Fig. 2a), 1 (Fig. 2b) or 2 (Fig. 2c), because the primary tumor had been inoculated 7, 8 or 9 days, respectively, before that day (Fig. 1 shows that tumor volume on day 9 was larger than that on day 8 and this was in turn larger than that on day 7 of tumor growth).

That is, the secondary tumor growth could be either stimulated or inhibited, apparently depending on the ratio between the mass of the primary tumor relative to that of the second tumor implant, with high ratios (secondary tumor implanted on day 3 onwards) rendering inhibition, low ratios (secondary tumor implanted on day 1) inducing stimulation and intermediate or very low ratios (secondary tumor implanted on day 2 or 0, respectively) producing no effect on secondary tumor growth.

A hormetic or biphasic dose–response curve describing these observations is depicted in Fig. 3.

Although the above-mentioned experiments suggest the hypothesis that inhibition or stimulation of secondary tumor implants depend on the relative tumor sizes between primary and secondary tumors, it remains only an assumption, since other changes might well take place with the passage of time other than or in addition to changes in tumor size. To further support that hypothesis, mice bearing a primary tumor in the right flank (initiated with an s.c. inoculum of $1 \times 10^6$ LB tumor cells) received, at a constant time (6 days) after the primary tumor inoculation, a variable number of secondary inoculations in the left flank ($1 \times 10^5$, $1 \times 10^6$ or $1 \times 10^7$ LB tumor cells) and the growth of the secondary tumor was registered. As shown in Fig. 4, while secondary tumor implants of $1 \times 10^5$ and

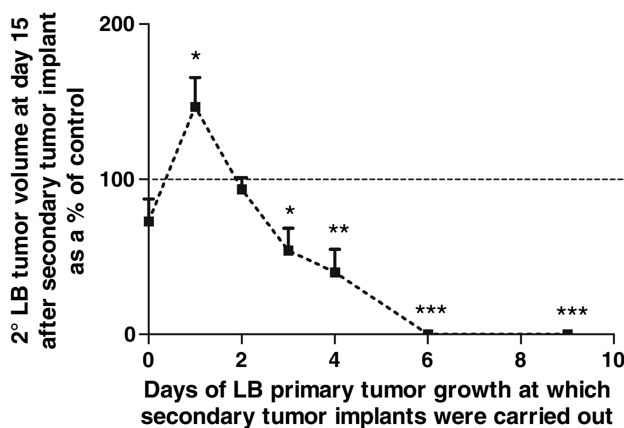


**Fig. 3** Hormetic curve summarizing the stimulatory and inhibitory effects induced by a primary LB tumor implanted in the right flank ($1 \times 10^6$ tumor cells) on the growth of secondary LB tumor implanted ($1 \times 10^5$ tumor cells) in the left flank, on selected days of primary tumor growth. *Dotted line* represents the tumor volume in control mice only receiving the tumor implant in the left flank. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

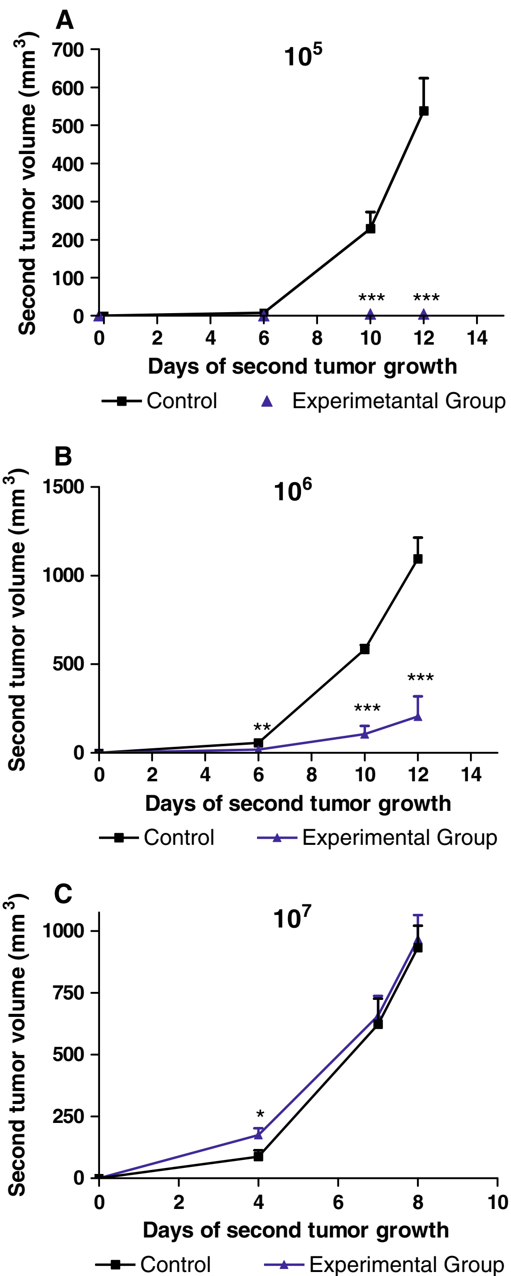


**Fig. 4** A total of 23 mice received an s.c. implant of $1 \times 10^6$ LB cells in the right flank on day 0. Six days later (day 6), mice were s.c. challenged with $1 \times 10^5$ ($n = 6$) (**a**); $1 \times 10^6$ ($n = 6$) (**b**) or $1 \times 10^7$ ($n = 11$) (**c**) LB cells in the left flank. Controls only received $1 \times 10^5$ ($n = 6$), $1 \times 10^6$ ($n = 6$) or $1 \times 10^7$ ($n = 15$) LB cells in the left flank. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

$1 \times 10^6$ LB tumor cells were completely and partially inhibited, respectively, as compared with the corresponding controls, the growth of $1 \times 10^7$ LB tumor cells was enhanced, especially during the first days of growth. Four days after the secondary implant with $1 \times 10^7$ LB tumor cells, the histological examination of the enhanced tumors revealed a higher number of both mitosis (mean number per HPF ± SE = 2.02 ± 0.13; $n = 50$ fields, ×1,000 magnification) and

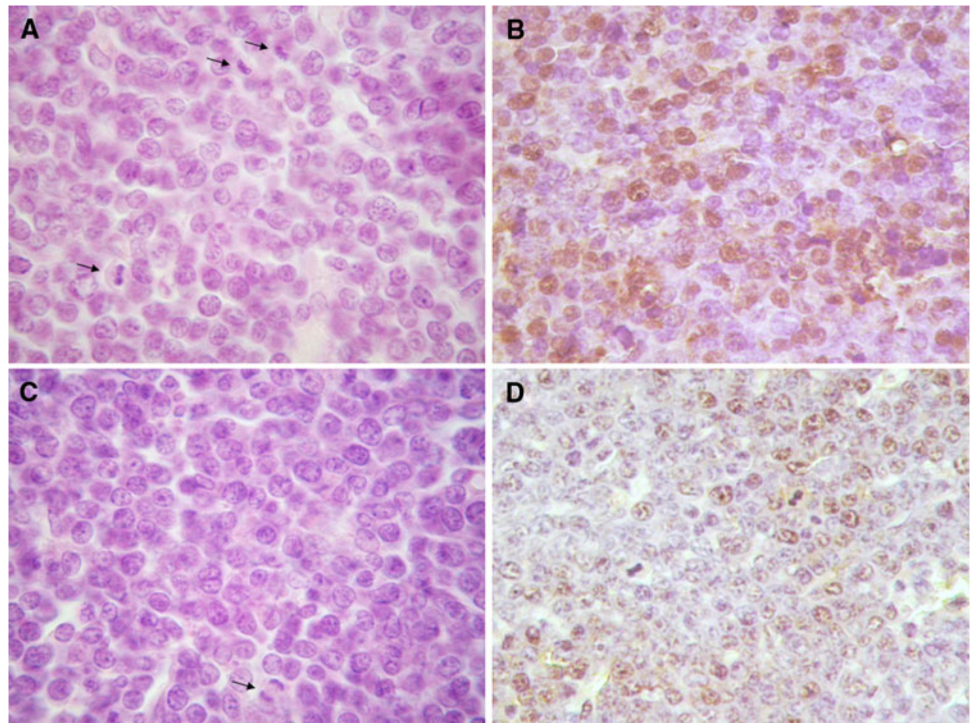


**Fig. 5** (**a–b**) Experimental group: 11 mice were inoculated s.c. on day 0 with $1 \times 10^6$ LB tumor cells in the right flank. On day 6, they were challenged with $1 \times 10^7$ LB tumor cells in the left flank. Four days later, the underlying secondary enhanced tumor from the left flank was examined. **a** Tumor cells with numerous mitotic figures (*arrows*), ×1,000. **b** Presence of abundant tumor cells displaying intense Ki-67 nuclear expression, ×400. (**c–d**): Control group: 15 mice received on day 6, $1 \times 10^7$ LB tumor cells only in the left flank. Four days later, the underlying tumor was processed as in the experimental group. **c** Tumor cells with few mitotic figures (*arrows*), ×1,000. **d** The presence of relatively few cells exhibiting slight Ki-67 nuclear expression, ×400

tumor cells expressing the proliferating nuclear protein Ki-67 (mean number per HPF ± SE = 111.22 ± 2.21; $n = 50$ fields, ×400 magnification) than that displayed by the controls only receiving the implant with $1 \times 10^7$ LB tumor cells in the left flank: 1.01 ± 0.09 mitosis per HPF; $n = 50$ fields; $p < 0.001$ and 59.74 ± 1.49 cells expressing Ki-67 per HPF; $n = 50$ fields; $p < 0.001$. Figure 5 illustrates these differences.

It is worth noting that, in all the experiments reported above, the primary tumor growth itself (initiated with an s.c. inoculum of $1 \times 10^6$ LB cells) was not affected by the presence of secondary tumor implants relative to the growth, in control animals, of a single s.c. inoculum of $1 \times 10^6$ LB cells (data not shown).

## Kinetics of a primary CEI tumor

The growth of a CEI tumor, initiated with an s.c. inoculum of $1 \times 10^6$ CEI cells, is plotted in Fig. 6. The tumor becomes initially detectable around day 12 and then its size increases progressively killing the host in about 50 days.

## Biphasic effect of a primary CEI tumor on the growth of secondary CEI tumor implants

A total of 43 mice received an s.c. implant of $1 \times 10^6$ CEI tumor cells in the right flank on day 0 (primary tumor) and then a secondary s.c. implant of $3 \times 10^5$ CEI cells in the left flank on days 2 ($n = 6$ mice), 6 ($n = 5$), 10 ($n = 4$), 16 ($n = 6$), 22 ($n = 15$) or 29 ($n = 7$), when the primary tumor volume was 0, 0, 0, 334, 658 and 1,665 mm$^3$, respectively. Twelve (12) mice that only received the tumor implant in the left flank served as control.

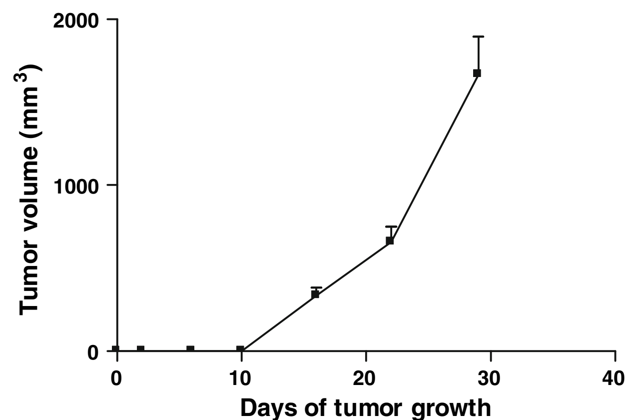


**Fig. 6** Growth of s.c. CEI tumor initiated with an inoculum of $1 \times 10^6$ LB cells in the right flank. Each point represents the mean ± SE of 12 mice

As shown in Fig. 7 the primary tumor produced different effects on the second tumor growth, depending on the day on which the second implant was carried out. In effect, when a second tumor was implanted on days 2 or 10, growth of the secondary tumor was not different from the control. When the second implant was carried out on day 6, the tumor was significantly enhanced. Finally, when the second tumor was implanted on day 16 onwards, a significant inhibition was observed. The most profound inhibition

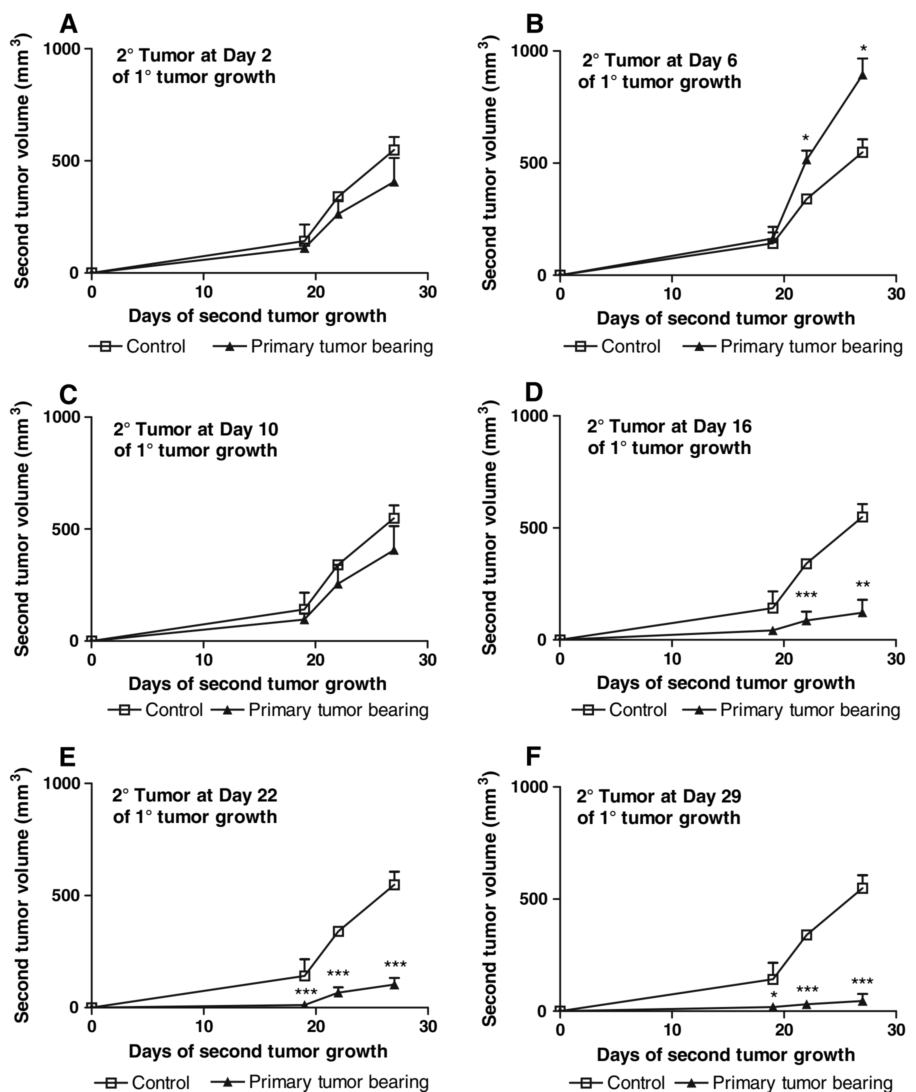


**Fig. 7** Growth of a secondary CEI tumor implant in primary CEI tumor-bearing mice. The primary CEI tumor was initiated with $1 \times 10^6$ tumor cells inoculated in the right flank on day 0. A secondary tumor was implanted ($3 \times 10^5$ CEI tumor cells) in the left flank on days 2 ($n = 6$) (**a**), 6 ($n = 5$) (**b**), 10 ($n = 4$) (**c**), 16 ($n = 6$) (**d**), 22 ($n = 15$) (**e**) or 29 ($n = 7$) (**f**) of primary tumor growth. Controls were 12 mice receiving only the inoculum of $3 \times 10^5$ CEI cells in the left flank. *$p < 0.02$, **$p < 0.01$, ***$p < 0.001$

was detected when the second tumor was implanted on day 29, that is, when the primary tumor was the largest.

Although on days 2, 6 and 10, no primary tumor mass was macroscopically detected, the mass of the primary tumor on the day when the second tumor began to be perceptible (day 19) was different in the cases in which the second tumor was implanted on days 2 (Fig. 7a), 6 (Fig. 7b) or 10 (Fig. 7c) because the primary tumor had been inoculated 21, 25 or 29 days, respectively, before that day (Fig. 6 shows that the tumor volume on day 29 was larger than that on day 25 and this in turn was larger than that on day 21 of tumor growth).

That is, as in LB, secondary tumor growth was either stimulated or inhibited depending on the ratios between the mass of the primary tumor relative to that of the second tumor implant, with high ratios (secondary tumor implanted on day 16 onwards) rendering inhibition, low ratios (secondary tumor implanted on day 6) inducing stimulation and intermediate and very low ratios (secondary tumor implanted on days 10 or 2, respectively) producing no effect on secondary tumor growth.

A hormetic dose–response curve describing these observations is depicted in Fig. 8.

It is worth noting that, in these experiments, the primary CEI tumor was not affected by the presence of secondary tumor implants (data not shown).

## Biphasic effect of serum from tumor-bearing mice on the in vitro proliferation of tumor cells

As anticipated by some studies (Meiss et al. 1986; Ruggiero et al. 1985), histological analysis showed that the effects of the non-immunogenic LB and CEI primary tumors on the growth of secondary tumor implants operated without the local participation of host cells: no lymphocytes, macrophages, neutrophils or other host cells were detected in the periphery or within the secondary tumor implants. Further, by using a cell-mediated cytotoxicity assay, spleen cells

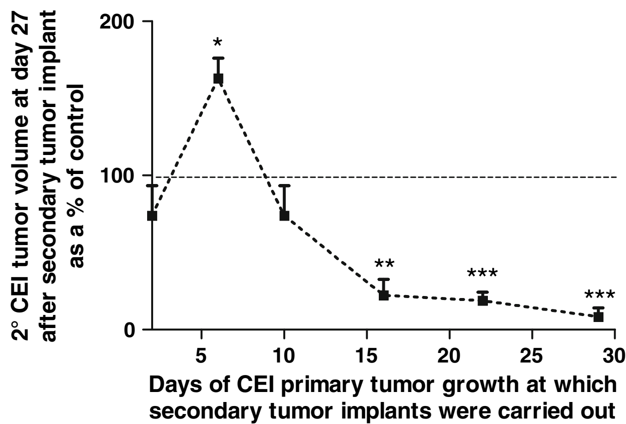


**Fig. 8** Hormetic curve summarizing the stimulatory and inhibitory effects induced by a primary CEI tumor implanted in the right flank ($1 \times 10^6$ tumor cells) on the growth of secondary CEI tumors implanted ($3 \times 10^5$ tumor cells) in the left flank, at selected days of primary tumor growth. *Dotted line* represents the tumor volume in control mice only receiving the tumor implant in the left flank. *$p < 0.02$, **$p < 0.01$, ***$p < 0.001$

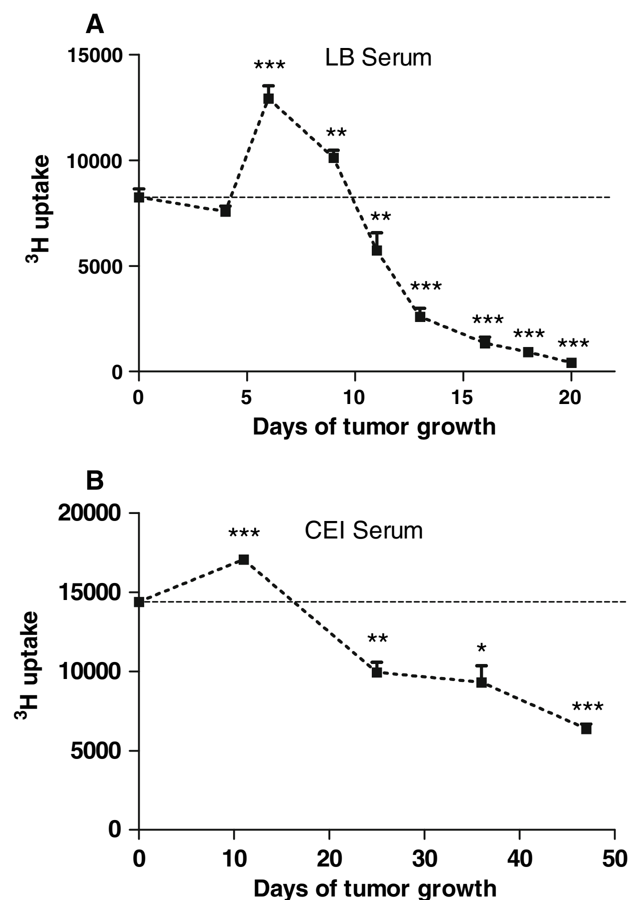


**Fig. 9** Tumor stimulatory and inhibitory activities of serum from **a** LB tumor-bearing mice (LB serum) or **b** CEI tumor-bearing mice (CEI serum) depending on the day of tumor growth on which the serum was collected. Values represent the mean ± SE of [$^3$H] thymidine uptake by LB tumor cells cultured with 25% of LB serum ($n = 6$–8 assays per day) or by CEI tumor cells cultured with 25% of CEI serum ($n = 5$). *Dotted line* represents the [$^3$H] thymidine uptake by LB (**a**) or CEI (**b**) tumor cells cultured with 25% of normal serum. *$p < 0.02$, **$p < 0.01$, ***$p < 0.001$

from LB and CEI tumor-bearing mice did not exhibit any differential effect on $^{51}$Cr-labeled LB or CEI tumor cells, as compared with normal spleen cells, independent of the day of tumor growth on which spleen cells were collected (data not shown).

On the other hand, serum from LB or CEI- tumor-bearing mice exhibited null, inhibitory or stimulatory effects on in vitro LB or CEI cell proliferation, depending on the day of tumor growth on which serum was collected. As for LB tumor (see Fig. 9a), while serum from day 4 behaved as normal serum, serum from days 6 or 9 produced a slight, but significant, stimulation as compared with normal serum. Finally, serum from day 11 onwards produced a strong inhibitory effect, proportional to tumor size. Similar observations were made with serum from CEI tumor-bearing mice (Fig. 9b).

In all cases (Fig. 9a, b), the effects of a low dilution (1/4) of serum were registered. When different dilutions of LB serum from day 20 (the most inhibitory serum) were assayed, we observed that while relatively low dilutions (1/2–1/16) displayed a profound inhibitory effect on in vitro tumor cell proliferation, higher dilutions (1/64) exhibited a slight but significant stimulatory effect, similar to that observed with low dilutions of serum from day 6. In Table 1, a representative experiment is shown. When LB serum from day 20 was subjected to dialysis, both the inhibitory and the stimulatory were recovered in the dialyzable fraction. Both activities were also recovered together in fractions of a G-15 Sephadex column corresponding to MW below 1,000 Da. As with the whole LB serum, low dilutions of these fractions exhibited a strong inhibitory effect on tumor cell proliferation, while high dilutions (in general, 1/64) displayed a slight, but significant, stimulatory effect: ratio of $^3$[H] thymidine uptake by tumor cells incubated with the low molecular weight fraction of LB serum related to the same dilution of normal serum: $1.25 \pm 0.12\%$; $n = 7$ experiments; $p < 0.05$. These observations raise the possibility that a factor of low MW displaying dual effects on tumor cell proliferation is present in the serum of tumor-bearing mice, the concentration of which increases throughout tumor growth with low concentrations rendering tumor stimulation and high concentrations producing tumor-inhibitory effects.

## Discussion

The hormetic dose–response can be described as stimulation in the low-dose zone, followed by an inhibitory

**Table 1** Effect of different dilutions of serum from LB tumor-bearing mice (LB serum, 20 days of tumor growth) on in vitro LB tumor proliferation

| Dilutions | $^{3}$[H]-Thymidine uptake by LB tumor cells (cpm ± SE) | | | |
|---|---|---|---|---|
| | Normal serum ($n$ = 6 per each dilution) | LB serum ($n$ = 6 per each dilution) | Percentage of normal serum | $p$ |
| 1/2 | 4,264 ± 206 | 461 ± 36 | 11 | <0.001 |
| 1/4 | 6,425 ± 250 | 921 ± 64 | 14 | <0.001 |
| 1/8 | 9,851 ± 317 | 1,842 ± 177 | 19 | <0.001 |
| 1/16 | 11,584 ± 454 | 4,221 ± 168 | 23 | <0.001 |
| 1/32 | 12,219 ± 428 | 7,832 ± 50 | 42 | <0.001 |
| 1/64 | 13,776 ± 824 | 18,184 ± 1346 | 132 | <0.02 |
| 1/128 | 14,229 ± 933 | 14,112 ± 654 | 99 | NS |
| 1/256 | 14,967 ± 838 | 14,667 ± 821 | 98 | NS |

response at higher doses. Although through nearly the past century, the concept of hormesis (originally called the Arndt-Schulz law) became marginalized in the biomedical literature because of its historical association with the medical practice of homeopathy, the last few years have witnessed growing evidence of hormetic dose responses occurring in many biological models, characterized by similar underlying features and explanatory strategies (Calabrese 2005). The phenomenon of hormesis has been observed not only in animal models, but also in plants. For example, high concentrations of auxin inhibit root growth, whereas low concentrations stimulate root development (Bertin et al. 2007).

In this study, by using two murine tumors of spontaneous origin and undetectable immunogenicity, we have explored the possibility that the hormesis concept may also describe the interactions between two tumors implanted in a single organism, such that, either by the relative timing of the inoculations or by using different number of tumor cells per inoculum, the resulting tumors are of different sizes. In such cases, we have found that the larger primary tumor induced inhibitory or stimulatory effects on the growth of the smaller secondary one, depending on the ratio between the mass of the larger tumor relative to that of the smaller one, with high ratios rendering inhibition and low ratios inducing stimulation of the secondary tumor. Very low ratios do not induce any effect. All of these observations can appropriately be described by a classical hormetic dose–response curve.

Furthermore, the magnitude of that stimulation, as measured by the increase of the secondary tumor relative to the growth observed in control animals receiving a single inoculum, was about 47–67% above control values depending on the tumor tested and the day after the secondary tumor implant. This relatively modest stimulation is similar to that observed in almost all cases of hormesis and in fact is one of its most distinguishing characteristics (Calabrese 2008a, b). On the other hand, in the experiments reported in this work, we have not observed any effect induced by the smaller secondary tumor on the larger primary tumor, presumably because, as we pointed above, no effect is observed below a critical low ratio. Some years ago, we reported a possible exception to this contention (Meiss et al. 1986), but it remains only an assumption because we were not able to reproduce that result herein.

The mechanism by which a non-immunogenic tumor affects the growth of a smaller one, both inoculated in a single animal, may be associated with that invoked as the cause of concomitant resistance induced by non-immunogenic tumors. Concomitant resistance is the phenomenon according to which a tumor-bearing host inhibits or retards the growth of a secondary tumor implant at a distant site. In previous studies, we demonstrated that concomitant resistance induced by non-immunogenic tumors was not associated with cellular effectors infiltrating the site of the secondary tumor implant, but with a low molecular weight serum factor different from antibodies and complement and unrelated to previously characterized growth inhibitors, such as interferons, tumor necrosis factors, the transforming growth factor beta family, angiostatin, endostatin, etc. (Franco et al. 1996; Ruggiero et al. 1990). In this study, we have suggested that this factor may induce both, inhibitory or stimulatory, effects on the in vitro proliferation of tumor cells, depending on its serum concentration, with high concentrations rendering inhibition and low concentrations inducing tumor stimulation.

The existence of putative tumor-enhancing and tumor-inhibitory factors that would be produced or induced by the tumor itself has been suggested or suspected from the study of Laird (1969), which showed that the growth of a wide variety of tumors followed the Gompertzian curve, with an early acceleration of tumor growth followed by an exponential decline in rate, late during tumor development. The latter was not caused by failure of blood supply or any other artifact of increased size.

On the basis that the concentration of the serum factor associated with the phenomenon of concomitant resistance

is proportional to tumor size, we can offer a putative explanation for the results in vivo reported in this study.

In the first place, we might understand why identical inocula of tumor cells secondarily implanted in tumor-bearing mice can be stimulated or inhibited, depending on the day of primary tumor growth on which the second inoculum was carried out. In effect, a secondary tumor implanted early during primary tumor growth, may face a low (stimulatory) concentration of that serum factor, so that it may be enhanced. In contrast, a secondary tumor implanted late during primary tumor growth will face a high, inhibitory concentration of the serum factor and in consequence will be inhibited.

In the second place, we might also understand why a variable inoculum of tumor cells, secondarily implanted at a constant time after the primary tumor inoculation, may be stimulated or inhibited depending on the size of the secondary inoculum. In effect, a large secondary tumor implant will be vascularized earlier during primary tumor growth than a small secondary one implanted simultaneously with the former. Therefore, the large tumor implant might face a low (stimulatory) concentration of the serum factor and in consequence its growth might be enhanced, at least up to the time when the serum factor reaches a high, inhibitory concentration. On the other hand, the small secondary implant would be vascularized relatively late during primary tumor growth; therefore, it would face, from the beginning, a high (inhibitory) concentration of the serum factor, and in which case would be inhibited.

It is plausible that the biphasic effect displayed by this serum factor may be explained, at least in part, according to the hypothesis of Szabadi (Szabadi 1977), which is able to account for many cases of hormetic-like biphasic dose–response relationships. Szabadi postulated that a single agonist (in this case, the serum factor of low molecular weight) might bind to two receptor subtypes, with one activating a stimulatory pathway while the other an inhibitory one. The receptor subtype with the greatest affinity for the serum factor would have fewer receptors on the target tumor cells and its pathway activation would dominate at low doses. On the other hand, the second receptor subtype, associated with the inhibitory pathway, would have lower agonist affinity but more receptors per cell, and in consequence it would become dominant at higher concentrations.

The characterization of the serum factor related to concomitant resistance and its receptor subtypes will be necessary to demonstrate if this generalized scheme proposed by Szabadi, can explain the biphasic effect of a primary tumor on the growth of secondary tumor implants. Taking into account that metastases can be considered as natural secondary tumor implants in a tumor-bearing host and that they constitute the main problem in cancer pathology (Bonfil et al. 1988; Farma et al. 2005; Loberg et al. 2007; Mimori et al. 2009; Ruggiero and Bustuoabad 2006), the use of the concept of hormesis to describe those biphasic effects might have significant clinical implications. For example, the definition of the windows of stimulation and inhibition in the relationship between the primary tumor and its metastases might help physicians to decide, in each particular case, the best therapeutic strategy, taking into account that if the tumor-bearing host were placed in the inhibitory window, tumor extirpation could enhance the growth of distant metastases and, reciprocally, if it were in the stimulatory window, tumor extirpation would result not only in a reduction or elimination of primary tumor load but also in a slower growth or inhibition of metastases.

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