J. Steroid Biochem. Molec. Biol. Vol. 37, No. 6, pp. 943–950, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

COMBINATION THERAPY WITH FLUTAMIDE AND MEDICAL (LHRH AGONIST) OR SURGICAL CASTRATION IN ADVANCED PROSTATE CANCER: 7-YEAR CLINICAL EXPERIENCE

F. LABRIE, A. DUPONT, L. CUSAN, J. GOMEZ, J. EMOND and G. MONFETTE
MRC Group in Molecular Endocrinology, CHUL Research Center and Laval University, 2705 Laurier Boulevard, Quebec, Canada G1V 4G2

Summary—Three hundred and sixty-three patients with clinical stage D2 prostate cancer who had not received previous endocrine therapy or chemotherapy were treated with the combination therapy using the pure antiandrogen Flutamide and the LHRH agonist [D-Trp^6,des-Gly-NH$_2^{10}$]LHRH ethylamide (or orchiectomy) for an average of 771 days (24–2607 days). Only 31 of the 308 evaluable patients (10.1%) did not show an objective positive response at the start of the combination therapy compared with an average of 18% in five recent studies using monotherapy. The median survival achieved using monotherapy is approximately 24 months while, in the present study, it is increased to 41.2 months, thus giving an additional 17 months of survival with the combination therapy. It should be mentioned that at the time of relapse, combination therapy is continued and, in addition, further blockade of adrenal androgen secretion is achieved with aminoglutethimide and hydrocortisone. While our studies showing the advantages of combination therapy with pure antiandrogen in advanced prostate cancer have been confirmed by independent large-scale randomized studies, our preliminary data clearly suggest the interest of downstaging early stage prostate cancer by temporary combination therapy prior to radical prostatectomy.

INTRODUCTION

Cancer of the prostate has become the most frequent cancer in men. It is estimated that 109,000 new cases of prostate cancer will be diagnosed in the United States in 1990 [1]. Prostate cancer is thus discovered at the rate of more than 300 new cases per day in North America alone. In more than 50% of patients, prostate cancer is at a very advanced stage (stage D2) and has already spread outside the prostate, usually into the bones, at the time of first diagnosis. Since the original observation of Huggins and his colleagues in 1941 [2] on the role of androgens of testicular origin, the standard therapy of advanced prostate cancer has been surgical castration or blockade of androgen formation by the testes with high doses of estrogens. Following these two approaches, a temporary response is observed in 60–80% of patients while 20–40% of subjects do not show any improvement in their disease following the start of treatment. Moreover, 50% of patients who initially benefit from an initial positive response show reappearance of the cancer within 1 yr. In addition, when relapse of the cancer occurs, the prognosis is poor and 50% of the patients are expected to die within the following six months [3].

When, for medical or psychological reasons, patients do not accept surgical castration, an extremely well tolerated and equally efficient means of eliminating testicular androgens is now available, namely the superagonists of luteinizing hormone-releasing hormone (LHRH) [4, 5]. Serum testicular androgens are thus easily reduced to castration levels during chronic treatment of men with LHRH agonists. The only side effects observed are those related to the blockade of testicular androgens, namely hot flushes and a decrease or loss of libido and potency in 75% of patients. These side effects are not greater than those already observed after orchiectomy.

However, it should be mentioned that although LHRH agonists offer a more acceptable method of castration free of important side effects, one cannot expect to improve the prognosis of prostate cancer beyond the results previously achieved with orchiectomy since the effect of LHRH agonists is also limited to the blockade of testicular androgens. Moreover,

Proceedings of the 2nd International EORTC Symposium on "Hormonal Manipulation of Cancer: Peptides, Growth Factors and New (Anti-)Steroidal Agents", Rotterdam, The Netherlands, 9–11 April 1990.

since LHRH agonists always induce a temporary rise of testosterone secretion during the first 5–12 days of treatment with the risk of tumor exacerbation or flare-up of the disease during this period [6, 7], these peptides should never be administered alone without the protection of simultaneous treatment with an antiandrogen [7, 8] which neutralizes the potentially harmful action of the LHRH agonist-induced elevation of circulating androgens.

In order to take into account the crucial observation that the adrenals contribute about 40% of total androgens responsible for the stimulation of prostate cancer growth in men [9], we have developed a combination therapy where the formation of androgens by the testes is blocked by the administration of an LHRH agonist or surgical castration while, at the same time, the action of androgens of adrenal origin is blocked by administration of the antiandrogen Flutamide. We present an update of our original study started in 1982 using the combination therapy in advanced prostate cancer.

PATIENTS AND METHODS

Starting in March 1982, 363 patients with histology-proven prostatic adenocarcinoma and bone metastases visualized by bone scintigraphy (stage D2) took part in this multicenter study conducted by the Laval University Prostate Cancer Program (LUPCP) in collaboration with investigators from 24 institutions across Canada and the United States. All patients were entered into the study after written informed consent. The mean duration of treatment was 771 days (24–2607 days). The last evaluation was on 30 September 1989. The criteria for inclusion and exclusion were those of the U.S. National Prostatic Cancer Project (NPCP) [10, 11] except that a life expectancy of at least 90 days and normal blood counts were not used as criteria for exclusion. All patients presenting with stage D2 prostate cancer and having received no previous endocrine therapy or chemotherapy were thus included. The patients with very advanced disease with a short life expectancy were not excluded in order to more closely mimick the situation found in usual urological practice.

Demographic data and baseline profiles for patients who received the combination therapy in the present study (LUPCP) and those of the Intergroup study [8] who received Leuprolide with or without Flutamide are shown in Table 1. In the present study, pain and abnormal performance were found in 229 (63%) and 158 (43%) patients, respectively. Location of metastases prior to combination therapy included bone metastases in all patients as well as involvement of lung (8, 5%), lymph nodes (58, 16%), bone marrow (11, 3%), brain (3, 0.8%) and liver (3, 0.8%).

Of the 363 previously untreated stage D2 patients, 353 received the combination treatment with the LHRH agonist (D-Trp^6,des-Gly-NH$_2^{10}$]LHRH ethylamide (Tryptex) in association with the pure antiandrogen 2-methyl-*N*-[4-nitro-3(trifluoromethyl)-phenyl]propanamide (Flutamide, Euflex, Eulexin) while 11 had

Table 1. Demographic data and baseline profiles for patients of the studies using combination therapy (LHRH agonist/ORCH + Flutamide) and LHRH agonist (Leuprolide) alone

Characteristic	Leuprolide + Placebo[a] (n = 300)	Leuprolide + Flutamide[b] (n = 303)	Tryptex/ORCH + Flutamide[b] (n = 363)
Mean age (yr) (range)	68 (46–98)	69 (44–86)	67 (38–86)
		(Percentage)	
ECOG[c] performance status			
0–2	94	93	94
3–4	6	7	6
Bone pain	78	77	63
Pathologic fractures	5	6	2
Severe disease	86	86	83
Regional nodal involvement	23	26	18
Distant nodal involvement	19	17	16
Lung or pleural involvement	8	6	5
Liver involvement	2	1	1
Metastatic bone involvement			
Pelvis	69	66	71
Rib	69	68	72
Vertebra	77	73	77
Long bone	46	38	49
Skull	30	27	29
Elevated acid phosphatase	80	78	88

[a] Crawford *et al.* [8].
[b] This study.
[c] ECOG, Eastern Cooperative Oncology Group.

orchiectomy (instead of LHRH agonist treatment). No difference in the clinical response was observed between chemical or surgical castration. Twenty patients were originally started randomly with the flutamide analog, 5,5-dimethyl-3-[4-nitro-3-trifluoromethyl)-phenyl]-2,4-imidazolidone (RU23908, Anandron). However, the occurrence of visual side effects in 70% of the patients receiving Anandron led to an early change from Anandron to Flutamide and the exclusive use of Flutamide in all patients since June 1983.

The LHRH agonist was injected subcutaneously at the daily dose of 500 μg at 0800 h for 1 month followed by a 250 μg daily dose while Flutamide was given 3 times daily at 0700, 1500 and 2300 h at the dose of 250 mg orally. The antiandrogen was started 2 h or 1 day before first administration of the LHRH agonist or orchiectomy. Recent kinetic data [12] and information about the rapid changes of sensitivity of androgen-sensitive tumors when exposed to partial blockade of androgens [13] indicate that the optimal time for first administration of Flutamide should be 2 h before first injection of the LHRH agonist or orchiectomy. This schedule has been used since June 1988. At the time of relapse under combination therapy, the treatment with Flutamide and [D-Trp^6,des-Gly-NH$_2^{10}$]LHRH ethylamide is continued. Moreover, in order to further block adrenal androgen secretion, aminoglutethimide is administered routinely at the dose of 250 mg every 8 h in association with a low dose of hydrocortisone acetate (10 mg at 0700 h, 5 mg at 1500 h and 5 mg at 2100 h [14]. The tolerance to this additional therapy has generally been good.

Complete clinical, urological, biochemical and radiological evaluation of the patients was performed before starting treatment as described [9]. The initial evaluation included history, physical examination, bone scan, transrectal and transabdominal ultrasonography of the prostate, ultrasonography of the abdomen, chest roentgenogram and skeletal survey and sometimes computerized axial tomography (CAT) of the abdomen and pelvis as well as excretory urogram (IVP). Bone scans were evaluated by an independent group of radiologists unaware of the treatment of the patients. Performance status and pain were evaluated on a scale of 0–4. The follow up was as described [9], patients being evaluated at 3, 6, 12 months and every sixth months thereafter.

RESULTS

A positive objective response assessed according to the criteria of the NPCP has been obtained in 277 of 308 patients (90%), thus leaving only 10% of the patients with no response at the start of treatment (Fig. 1).

The percentage of complete responses obtained, namely 24.4%, can be compared to an average of only 4.6% in the five studies limited to a blockade of testicular androgens [15–17]. The rate of complete objective responses is thus increased by 5.3-fold (Fig. 1, Table 2). Another important observation is that only 10% of patients continued to progress after starting combination therapy while an average of 18%

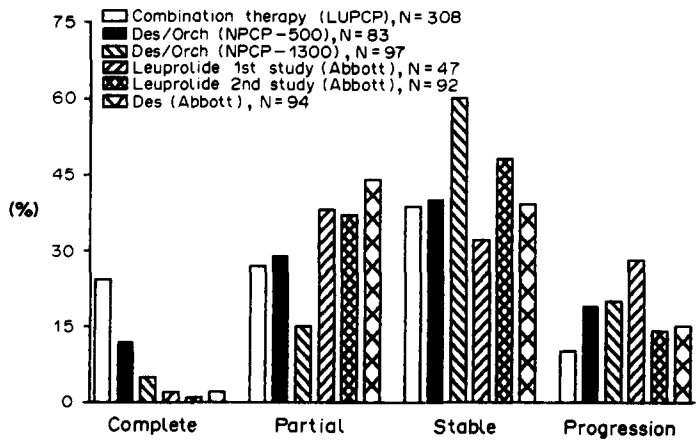

Fig. 1. Comparison of the best objective response rates assessed according to US NPCP criteria following combination therapy (LUPCP) and five studies using ORCH, DES or Leuprolide alone [15–17]. All were previously untreated patients with clinical stage D2 prostate cancer.

Table 2. Best objective response (U.S. NPCP criteria) achieved after combination therapy with Flutamide (LUPCP) compared with the results of orchiectomy or estrogens (NPCP-500, NPCP-1300, DES) and the LHRH agonist Leuprolide

Best response achieved	NPCP-500[a] ($n = 83$)	NPCP-1300[a] ($n = 97$)	Leuprolide-1[b] ($n = 47$)	Leuprolide-2[c] ($n = 92$)	DES[c] ($n = 94$)	LUPCP ($n = 363$)
Complete	10 (12%)	5 (5%)	1 (2%)	1 (1%)	2 (2%)	75 (24.4%)
Partial	24 (29%)	15 (15%)	18 (38%)	34 (37%)	41 (44%)	83 (26.9%)
Stable	33 (40%)	58 (60%)	15 (32%)	44 (48%)	37 (39%)	119 (38.6%)
Progression	16 (19%)	19 (20%)	13 (28%)	13 (14%)	14 (15%)	31 (10.1%)

[a] NPCP [16].
[b] Leuprolide-1-[17].
[c] Leuprolide-2-[15].

Table 3. Comparison of median survival times observed in the most recent studies using combination therapy and monotherapy

Studies	Disease-free survival (months)		Survival (months)	
	LHRH-A/ORCH	Combination therapy	LHRH-A/ORCH	Combination therapy
Crawford *et al.* [8]	13.9	16.5	28.3[a]	35.6
Anandron study [19, 20]	12	17	19	33
LUPCP		21.9		41.2
Leuprolide study [15]	13.8		(Not estimated)	
NPCP-500 [16]	11.6		21.2	
DES/ORCH				

[a] Flutamide was added at the time of progression.

of patients did not respond upon initiation of monotherapies (Fig. 1 and Table 2), thus representing a 1.8-fold difference in favor of combination therapy.

In the present study, the probability of continuing response is 82.6% at 1 yr, 53.7% at 2 yr, 38.4% at 3 yr, 28.0% at 4 yr and 22.9% at 5 yr. The median disease-free survival time estimated for the 308 evaluated patients is 21.9 months (Table 3). Such an improvement of disease-free survival is in agreement with the 5.3-fold increase in the number of complete responses associated with a 1.8-fold decrease of incidence in the non-responders.

As illustrated in Fig. 2, the median survival time was estimated at 41.2 months, the probabilities of survival at 1, 2, 3, 4, 5, 6 and 7 yr being 87.5, 71.5, 55.5, 45.1, 36.0, 28.8 and 26.1%, respectively. Comparison of the survival times observed following combination therapy and those recently obtained with DES, orchiectomy or LHRH agonists alone are shown in Table 3. When comparing the probabilities of death obtained with combination therapy, and those obtained in the EORTC study 30761 [18], a difference in the survival rate is already present during the first year of treatment (Fig. 3). In this figure, patients treated with different monotherapy modalities, namely cyproterone acetate, medroxyprogesterone acetate and DES were pooled together.

Clinical symptomatology, especially pain and performance status were rapidly and markedly improved with the combination therapy. Moreover, as already reported, the combination therapy using Flutamide and castration was well tolerated. The side effects observed are those due to hypoandrogenecity, namely hot flushes

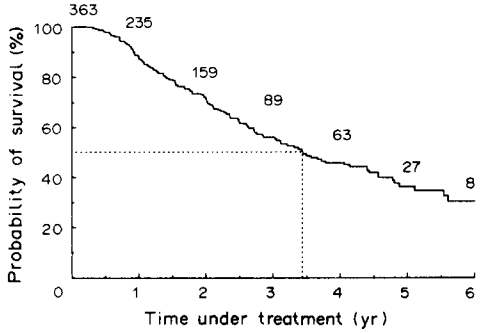

Fig. 2. Probability of survival following combination therapy (LUPCP study). Dotted line indicates the median survival. The numbers on the curve correspond to the number of patients at risk at each indicated time interval.

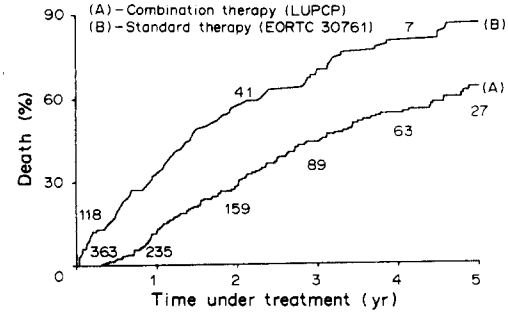

Fig. 3. Comparison of the probability of death following (A) combination therapy and (B) treatment with monotherapy (DES, cyproterone acetate or medroxyprogesterone acetate) [18].

and a decrease or loss of libido and sexual potency. Otherwise, the main complaint was loose bowel movements and diarrhea (9%).

DISCUSSION

It is well recognized that a positive correlation is observed between the incidence of the objective responses and survival [10, 16]. In fact, the patients who achieve a complete objective response have the best prognosis while those who achieve partial or stable response as best response have a shorter life expectancy [10]. On the other hand, the patients who show no response to treatment have a poor prognosis with a median life expectancy of only 6–9 months.

When compared with recent data obtained with monotherapy, the present study shows that combination therapy causes a 5.3-fold increase in the number of patients who achieved a complete response (from 4.8 to 24.4%) while the percentage of non-responders decreased from 18 to 10%. In addition to providing an improvement in the quality of life, the observed increase in the number of complete responses and a decrease in the proportion of non-responders, leads to 1.5 yr of additional survival. In the Intergroup study, the combination of complete and partial responders was 43% for combination therapy compared with 35% for monotherapy [8]. Similarly, in the Anandron study, 46% of patients achieved complete or partial responses in the orchidectomy + Anandron group while the same best response was obtained in only 20% of patients treated by orchidectomy and placebo [20]. In the same study, 38% of patients did not respond to orchiectomy alone while the percentage of non-responders decreased to 20% following combination therapy. In fact, all the randomized trials comparing medical or surgical castration in association with a pure antiandrogen (Flutamide or Anandron) with castration, and analyzed after a sufficiently long time, have shown the advantages of the combination therapy on: the best response achieved; the duration of response; and, even more importantly, on survival [8, 19, 20]. Since the advantages of the antiandrogen have been observed in combination with both orchiectomy [19–23] and LHRH agonists ([18] and this study), the benefits are not limited to prevention of flare of the disease during the first days of treatment with an LHRH agonist but are secondary to the inhibition by the antiandrogen of the action of the adrenal androgens.

It is now well recognized that the "apparently low" levels of plasma testosterone (T) and dihydrotestosterone (DHT) remaining after surgical or medical castration in men do not properly reflect the degree of inhibition of androgen action in target tissues [9, 24]. The main androgen precursors of adrenal origin in men are dehydroepiandrosterone (DHEA) (25 mg/day) and androstenedione (Δ^4-dione) 3 mg/day [25].

Man is unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids dehydroepiandrosterone (DHEA) and especially its sulfate (DHEA-S) which are converted into potent androgens in peripheral tissues. Adrenal secretion of DHEA and DHEA-S increases during the adrenarche in children at the age of 6–8 yr and very high values of circulating DHEA-S are maintained through adulthood [9, 26–29]. In fact, plasma DHEA-S levels in adult men are 100–500 times higher than those of testosterone.

This situation of a high secretion rate of precursor adrenal androgens in men is thus completely different from all animal models used in the laboratory, namely rats, mice, guinea-pigs, monkeys, or others, where the secretion of sex steroids takes place exclusively in the gonads ([9, 30] and Refs therein). These findings open a new field of endocrinology, namely that of intracrine secretion. Through intracrine activity, locally produced androgens and/or estrogens exert their action inside the same cells where synthesis takes place. This new section of endocrinology has recently been called intracrine activity [31], a terminology complementary to the well known autocrine, paracrine and endocrine activities where a hormone is active at the surface of the producing cells (autocrine), a hormone is acting on neighboring cells (paracrine) or a hormone released in the circulation is acting on distant target tissues (endocrine).

A major problem in this area which is likely to be at least partially responsible for the delayed progress is the fact that all animal models used in the laboratory, as mentioned above, do not secrete appreciable amount of adrenal precursor steroids, thus focusing all attention on the testes as the exclusive source of androgens for target tissue growth and function.

The most direct and straightforward evidence of an important role for adrenal androgens in

prostatic cancer is the finding that the active androgen dihydrotestosterone (DHT) remains at physiologically active concentrations in prostatic cancer tissue following removal of testis androgens by orchiectomy or treatment with estrogens or LHRH agonists. In fact, a high concentration of the active androgen, DHT, remains in prostatic cancer tissue following castration. Although orchiectomy, estrogens, or LHRH agonists (through blockade of release of bioactive LH) cause a 90–95% reduction in serum testosterone (T) concentration [4, 9, 32, 33], a much smaller effect is observed on the only meaningful parameter of androgenic action, namely the concentration of DHT in prostatic cancer tissue [24].

Measurements of T and DHT concentrations in serum have little or no value except as an index of testis activity. In fact, intraprostatic DHT concentration is the only significant parameter which indicates the level of androgenic activity at its site of action if prostatic cancer tissue.

As another measure of the importance of adrenal androgens in adult men, the serum levels of the main metabolites of androgens, namely 5α-androstane-3α,17β-diol (3α-diol), androsterone (ADT) and their glucuronidated derivatives, are only reduced by 50–70% [34, 35], thus reflecting the high level of adrenal precursors converted into DHT in castrated men.

In order to stimulate prostatic growth, the adrenal steroid precursors DHEA-S, DHEA, and Δ^4-dione must be taken up by the prostatic tissue and be locally metabolized into active androgens. That all three steroids can accumulate in human prostatic tissue has been well demonstrated by measurement of the three steroids in prostatic tissue removed at surgery for the treatment of benign prostatic hyperplasia, following infusion of the radioactive compounds [36]. Radioactive DHEA-S infused into patients is taken into prostatic tissue and transformed into DHEA, Δ^4-dione, androsterone (ADT), epiandrosterone (Epi-ADT), T and DHT [36]. Since DHEA-S is present at such high levels (500–5000 ng/ml) in the circulation, a small percentage of transformation of this steroid into DHT is sufficient to play a major role in the evolution of prostatic cancer.

It is now well demonstrated that the human prostate possesses all the enzymes required for the synthesis of active androgens from inactive adrenal steroid precursors, thus providing the explanation for the findings of intraprostatic concentrations of DHT as high as 1.5 ng/g tissue (4.5 nM) following surgical or medical castration [37]. With regard to the latter steroid, it might be relevant to recall that treatment with Flutamide (250 mg, every 8 h) decreases intraprostatic DHT levels below 0.2 ng/g tissue [38]. Such findings are indicative of the efficiency of Flutamide in displacing intraprostatic DHT from its intracellular receptor. Knowing that the K_d value of DHT interaction with the androgen receptor is less than 1 nM [12, 39], it is clear that a concentration of 1.5 nM DHT left in the prostate cancer tissue after castration will exert a major stimulatory effect on cancer growth.

Although the origin of tumors is believed to be monoclonal [40], it is clear that most, if not all, advanced tumors are composed of mixed populations of cells having a wide range of phenotypes. That heterogeneity of androgen sensitivity analogous to the one described in the Shionogi model [13, 41] exists in human prostate cancer is unequivocally demonstrated by the clinical data showing a 30–60% objective response to adrenalectomy, hypophysectomy, Flutamide or aminoglutethimide in patients who relapse after orchiectomy or treatment with estrogens [42–48]. Such a response to further androgen blockage in patients already castrated can only be explained by the presence in these patients of prostatic tumors which were still growing in the lower androgenic environment provided by the adrenal androgens remaining after medical or surgical castration. These patients were previously thought to have "androgen-resistant" tumors at start of treatment while, on the contrary, they have androgen-hypersensitive tumors. The approximately 10% of patients who do not respond to combination therapy might be bearing truly androgen-resistant tumors or, alternatively, these tumors could well be even more androgen-sensitive and able to grow with the small fraction of free androgens remaining free in their prostatic tumors in the presence of therapeutic doses of the antiandrogen and castration. Further blockade of adrenal androgen secretion and/or action will be needed to differentiate between these two possibilities.

In addition to the long-term beneficial effects of combination therapy, the use of Flutamide at the start of treatment eliminates the unnecessary risks of disease flare which are known to occur in a significant proportion of patients treated with an LHRH agonist alone [7, 33, 49–50]. With the undisputable knowledge that the

adrenals contribute 30–50% of total androgens in men and the positive results obtained in all studies using a pure antiandrogen (Flutamide or Anandron) in association with medical or surgical castration, it seems logical to suggest that combination therapy should be the standard treatment for all patients suffering from advanced prostate cancer.

REFERENCES

1. Silverberg E., Boring C. C. and Squires T. S.: Cancer Statistics, 1990. CA-A *Cancer J. Clin.* **40** (1990) 9–28.
2. Huggins C. and Hodges C. V.: Studies of prostatic cancer. I. Effect of castration, estrogen and androgen injections on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res.* **1** (1941) 293–297.
3. Johnson D. E., Scott W. W., Gibbons R. P., Prout G. R., Schmidt J. D., Chu T. M., Gaeta J., Sarrott J. and Murphy G. P.: National randomized study of chemotherapeutic agents in advanced prostatic carcinoma: progress report. *Cancer Treat. Rep.* **61** (1977) 317–323.
4. Labrie F., Bélanger A., Cusan L., Séguin C., Pelletier G., Kelly P. A., Reeves J. J., Lefebvre F. A., Lemay A., Goudreau Y. and Raynaud J. P.: Antifertility effects of LHRH agonists in the male. *J. Androl.* **1** (1980) 209–228.
5. Labrie F., Dupont A., Bélanger A., St-Arnaud R., Giguère M., Lacourcière Y., Emond J. and Monfette G.: Treatment of prostate cancer with gonadotropin-releasing hormone agonists. *Endocr. Rev.* **7** (1986) 67–74.
6. Waxman J., Man A., Hendry W. F., Whitfield H. N., Besser G. M., Tiptaft R. C., Paris A. M. I. and Oliver R. T. D.: Importance of early tumour exacerbation in patients treated with long-acting analogues of gonadotropin-releasing hormone for advanced prostatic cancer. *Br. Med. J.* **291** (1985) 1387–1388.
7. Labrie F., Dupont A., Bélanger A. and Lachance R.: Flutamide eliminates the risk of disease flare in prostate cancer patients treated with an LHRH agonist. *J. Urol.* **138** (1987) 804–806.
8. Crawford E. D., Eisenberger M. A., McLeod D. G., Spaulding J. T., Benson R., Dorr F. A., Blumenstein B. A., Davis M. A. and Goodman P. J.: A controlled trial of leuprolide with and without Flutamide in prostatic carcinoma. *New Engl. J. Med.* **321** (1989) 419–424.
9. Labrie F., Dupont A. and Bélanger A.: A complete androgen blockade for the treatment of prostate cancer. In *Important Advances in Oncology* (Edited by V. T. de Vita, S. Hellman and S. A. Rosenberg). J. B. Lippincott, Philadelphia (1985) pp. 193–200.
10. Labrie F., Dupont A., Giguère M., Cusan L., Bergeron N., Emond J., Monfette G., Lacourcière Y., Boucher H. and Lachance R.: Important prognostic value of standardized objective criteria of response in stage D2 prostatic carcinoma. *Eur. J. Cancer Clin. Oncol.* **12** (1988) 1869–1878.
11. Slack N. H., Murphy G. D. and NPCP Participants: Criteria for evaluating patient responses to treatment modalities for prostatic cancer. *Eurol. Clin. North Am.* **11** (1984) 337–342.
12. Simard J., Luthy I., Guay J., Bélanger A. and Labrie F.: Characteristics of interaction of the antiandrogen flutamide with the androgen receptor in various target tissues. *Molec. Cell. Endocr.* **8** (1986) 293–300.
13. Labrie F. and Veilleux R.: A wide range of sensitivities to androgens develops in cloned Shionogi mouse mammary tumor cells. *Prostate* **8** (1986) 293–300.
14. Labrie F., Dupont A., Bélanger A., Cusan L., Brochu M., Turina E., Pinault S., Lacourcière Y. and Emond J.: Anti-hormone treatment for prostate cancer relapsing after treatment with Flutamide and castration. *Br. J. Urol.* **63** (1989) 634–638.
15. The Leuprolide Study Group: Leuprolide versus diethylstilbestrol for metastatic prostate cancer. *New Engl. J. Med.* **311** (1984) 1281–1286.
16. Murphy G. P., Beckley S., Brady M. F., Chu M., Dekernion J. B., Dhabuwala C., Gaeta J. F., Gibbons R. P., Loening S. A., McKiel C. F., McLeod D. G., Pontes J. E., Prout G. R., Scardino P. T., Schlegel J. U., Schmidt J. D., Scott W. W., Slack N. H. and Soloway M.: Treatment of newly diagnosed metastatic prostate cancer patients with chemotherapy agents in combination with hormones versus hormones alone. *Cancer* **51** (1983) 1264–1272.
17. Smith J. A., Glode L. M., Wettlaufer J. N., Stein B. S., Glass A. G., Max D. T., Anbar D., Jagst C. L. and Murphy G. P.: Clinical effects of gonadotropin-releasing hormone analogue in metastatic carcinoma of the prostate. *Urology* **20** (1985) 106–112.
18. Pavone-Macaluso M., De Voogt H. J., Viggiano G., Barasolo E., Hardennois B., De Pauw M. and Silvester R.: Comparison of diethylstilbestrol, cyproterone acetate and medroxyprogesterone acetate in the treatment of advanced prostatic cancer: final analysis of the randomized phase III trial of the European Organization for Research on Treatment of Cancer Urological Group. *J. Urol.* **36** (1986) 624–631.
19. Raynaud J. P.: Antiandrogens in combination with LHRH agonist in prostate cancer. *Am. J. Clin. Oncol.* **II** (Suppl. 2) (1988) S132–S147.
20. Béland G., Elhilali M., Fradet Y., Laroche B., Ramsey E. W., Trachtenberg J., Venner P. M. and Tewari H. D.: Total androgen blockade versus castration in metastatic cancer of the prostate. In *Hormonal Therapy of Prostatic Diseases: Basic and Clinical Aspects* (Edited by M. Motta and M. Serio). Medicom, Bussum (1988) pp. 302–311.
21. Brisset J. M., Boccon-Gibod L., Botto H., Camey M., Cariou G., Duclos J. M., Duval F., Gontiès D., Jorest R., Lamy L., Le Duc A., Mouton A., Petit M., Praweman A., Richard F., Savatowsky I. and Vallancien G.: Anandron (RU23908) associated to surgical castration in previously untreated Stage D prostate cancer: a multicenter comparative study of two doses of the drug and of a placebo. *Progress. Clin. Biol. Res.* **243** (1987) 411–422.
22. Namer M., Amiel J. and Toubol J.: Anandron (RU23908) associated with orchiectomy in stage D prostate cancer: preliminary results of a randomized double-blind study. *Am. J. Clin. Oncol.* **11** (Suppl. 2) (1988) S191–S196.
23. Navratil H.: Double-blind study of Anandron versus placebo in stage D2 prostate cancer patients receiving buserelin. *Progress Clin. Biol. Res.* **243A** (1987) 401–410.
24. Geller J., Albert J., Nachtseim D. A. and Loza D. C.: Advantages of total androgen blockade in the treatment of advanced prostate cancer. *Semin. Oncol.* **15** (1988) 53–61.
25. Sanford E. J., Paulson D. F., Rohner T. J., Drago J. R., Santen R. J. and Bardin C. W.: The effects of castration on adrenal testosterone secretion in men with prostatic carcinoma. *J. Urol.* **118** (1977) 1019–1021.
26. de Peretti E. and de Forest M. G.: Pattern of plasma dehydroepiandrosterone sulfate levels in human from birth to adulthood. Evidence for testicular production. *J. Cell Endocr. Metab.* **47** (1978) 572–577.

27. Cutler G. B. Jr, Glenn M., Bush M., Hodgen G. D., Graham C. E. and Loriaux D. L.: Adrenarche: a survey of rodents, domestic animals and primates. *Endocrinology* **103** (1978) 2112–2118.
28. Adams J. B.: Control of secretion and the function of C19-delta-5-steroids of the human adrenal gland. *Molec. Cell Endocr.* **45** (1985) 1–17.
29. Brochu M. and Bélanger A.: Increase in plasma steroid glucuronide leels in men from infancy to adulthood. *J. Cell Endocr. Metab.* **64** (1987) 1283–1287.
30. Bélanger B., Bélanger A., Labrie F., Dupont A., Cusan L. and Monfette G.: Comparison of residual C-19 steroids in plasma and prostatic tissue of human, rat and guinea pig after castration: unique importance of extratesticular androgen in men. *J. Steroid Biochem.* **32** (1989) 695–698.
31. Labrie C., Bélanger A. and Labrie F.: Androgenic activity of dehydroepiandrosterone and androstenedione in the rat ventral prostate. *Endocrinology* **123** (1988) 1412–1417.
32. Warner B., Worgul T. J., Drago J., Demers L., Dufau M., Max D. and Santen R. J., Abbott Study Group: Effect of very high doses of D-leucine6-gonadotropin-releasing hormone proethylamide on the hypothalamic-pituitary testicular axis in patients with prostatic cancer. *J. Clin. Invest.* **71** (1973) 1842–1855.
33. Waxman J. H., Was J. A. H., Hendry W. F., Whilfield H. N., Besser G. M., Malpas J. S. and Olivier R. T. D.: Treatment with gonadotropin-releasing hormone analogue in advanced prostatic cancer. *Br. Med. J.* **286** (1983) 1309–1312.
34. Moghissi E., Alban F. and Horton R.: Origin of plasma androstanediol glucuronide in men. *J. Clin. Endocr. Metab.* **59** (1984) 417–421.
35. Bélanger A., Brochu M. and Cliche J.: Levels of plasma steroid glucuronides in intact and castrated men with prostatic cancer. *J. Clin. Endocr. Metab.* **62** (1986) 812–815.
36. Harper M. E., Pike A., Peeling W. B. and Griffiths K.: Steroids of adrenal origin metabolized by human prostatic tissue both *in vivo* and *in vitro*. *J. Endocr.* **60**, (1974) 117–125.
37. Labrie F., Bélanger A., Veilleux R., Lacoste D., Labrie C., Marchetti B., Poulin R., Dupont A., Cusan L. and Luthy I.: Rationale for maximal androgen withdrawal in the therapy of prostate cancer. *Baillière's Clin. Oncol.* **2** (1988) 597–619.
38. Labrie F., Luthy I., Veilleux R., Simard J., Bélanger A. and Dupont A.: New concepts on the androgen sensitivity of prostate cancer. In *Proc. 2nd Int. Symp. on Prostate Cancer* (Edited by G. Murphy). Liss, New York (1987) pp. 145–72.
39. Asselin J., Mélançon R., Moachon G. and Bélanger A.: Characteristics of binding to estrogen, androgen, progestin and glucocorticoid receptors in 7,12-dimethylbenz(a)anthracene-induced mammary tumors and their hormonal control. *Cancer Res.* **40** (1980) 1612–1622.
40. Dexter D. and Calabresi P.: Intraneoplastic diversity. *Biochim. Biophys. Acta* **694** (1982) 97–112.
41. Luthy I. and Labrie F.: Development of androgen resistance in mouse mammary tumor cells can be prevented by the antiandrogen flutamide. *Prostate* **10** (1987) 89–94.
42. Murray R. and Pitt P.: Treatment of advanced prostatic cancer resistant to conventional therapy with aminoglutethimide. *Eur. J. Cancer Clin. Oncol.* **21** (1985) 453–58.
43. Maddy J. A., Winternitz W. W. and Norrell H.: Cryohypophysectomy in the management of advanced prostatic cancer. *Cancer* **28** (1971) 322–328.
44. Becker H.: Endocrine treatment of advanced prostatic cancer. *14th Int Cancer Congr.*, Budapest (1986) (Abstr. 3291).
45. Drago J. R., Santen R. J., Lipton A., Worgul T. J., Harvey H. A., Boucher A., Manni A. and Rohner T. J.: Clinical effect of aminoglutethimide, medical adrenalectomy, in treatment of 43 patients with advanced prostatic carcinoma. *Cancer* **53** (1984) 1447–1450.
46. Kreis W., Ahmann F. R. and Crawford E. D.: Clinical effects and hormone profiles in men with advanced prostate cancer under treatment with aminoglutethimide. *14th Int. National Cancer Congr.*, Budapest (1986) (Abstr. 4619).
47. Stoliar B. and Albert P. J.: SCH13521 in the treatment of advanced carcinoma of the prostate. *J. Urol.* **111** (1974) 803–807.
48. Labrie F., Dupont A., Giguère M., Borsanyi J. P., Lacourcière Y., Monfette G., Emond J. and Bergeron N.: Benefits of combination therapy with Flutamide in patients relapsing after castration. *Br. J. Urol.* **61** (1988) 341–346.
49. Kahan A., Delrieu F., Amor B., Chiche R. and Steg A.: Disease flare induced by D-Trp^6-GnRH analgoue in patients with metastatic prostatic cancer. *Lancet* **1** (1984) 971–972.
50. Labrie F., Dupont A., Bélanger A., Emond J. and Monfette G.: Simultaneous administration of pure antiandrogens, a combination necessary for the use of luteinizing hormone-releasing hormone agonists in the treatment of prostate cancer. *Proc. Natn. Acad. Sci. U.S.A.* **81** (1984) 3861–3863.