On the alleged anticancer efficacy of the phosphoethanolamine pill, weakness of scientific evidence and ethical concerns

Sobre a alegada eficácia anti-câncer da pílula de fosfoetanololamina, fragilidade da evidência científica e preocupações éticas

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ABSTRACT

Anecdotal reports say that cancer patients improved after taking “synthetic phosphoethanolamine” (syn-PEA), anticancer pills produced and distributed by chemists from a Brazilian university. Notwithstanding the fact that syn-PEA pill inventors disseminated in the lay press the information that their drug is effective against different types of malignant tumors, they showed no clinical documentation or case reports to corroborate this statement. Moreover, syn-PEA failed to exhibit a consistent anticancer response in in vitro assays with human and murine cancer cell lines, and in in vivo xenograft tumor rodent assays. Despite the lack of nonclinical and clinical evidence of drug efficacy and safety, a bill authorizing production, prescription and consumption of syn-PEA pill passed the Congress and the president signed it into law (Law 13269/2016) on April 13, 2016. Astonishingly, the National Committee for Ethics in Research approved (April 19, 2016) syn-PEA trials in cancer patients in the absence of scientifically valid indications of a probable efficacy and without an adequate preclinical safety evaluation. It is unlikely that syn-PEA will eventually play a role in cancer therapy. Nonetheless, syn-PEA sad story unavoidably damaged country’s reputation as far as drug regulation and human research ethical standards are concerned.

KEYWORDS: Preclinical Studies; Clinical Research Ethics; Anticancer Drug; Oncologic Drugs; Cancer

RESUMO

Tem sido informalmente relatado que pacientes com câncer melhoraram após tomar pílulas de fosfoetanololamina sintética (sin-FEA) produzidas e distribuídas por químicos de uma universidade brasileira. Embora os inventores da sin-FEA divulgem na imprensa leiga que o seu medicamento é eficaz contra diferentes tipos de tumores malignos, eles não apresentaram documentação clínica e relatos de caso que corroborem esta afirmação. Além disso, a sin-FEA não mostrou uma resposta anticarcinogênica consistente em ensaios in vitro com células neoplásicas humanas e murinas, e em testes in vivo em roedores com tumores transplantados. Apesar da falta de evidência não clínica e clínica de eficácia e segurança deste medicamento, uma lei autorizando a produção, prescrição e consumo da sin-FEA foi aprovada pelo Congresso e sancionada sem vetos pela presidente (Lei nº 13.269/2016) em 13 de abril de 2016. Surpreendentemente, a Comissão Nacional de Ética em Pesquisa aprovou (em 19 de abril de 2016) testes da sin-FEA em pacientes, apesar da ausência de indícios cientificamente válidos de provável eficácia e de adequada avaliação pré-clínica de segurança. É improvável que a sin-FEA seja útil no tratamento do câncer. Entretanto, a triste história da sin-FEA inevitavelmente maculou a reputação do país com respeito à regulação de medicamentos e padrões éticos de pesquisa clínica.

PALAVRAS-CHAVE: Estudos Pré-clínicos; Ética em Pesquisa Clínica; Medicamentos Antineoplásicos; Medicamentos Oncológicos; Câncer
INTRODUCTION

Despite anecdotal reports that cancer patients improved after taking phosphoethanolamine (PEA) pills, it remains obscure whether PEA has in fact any influence on the tumorigenesis process. Present in literally every animal tissue, PEA is formed by phosphorylation of ethanolamine (through an ethanolamine kinase-catalyzed reaction) and acts as an intermediate in the biosynthesis of phosphoglycerides and sphingomyelin that serve as components of cell membranes (Figure 1).

As early as in 1936, Edgar Outhouse2,3 found substantial amounts of PEA in bovine tumors and speculated that this primary amine would be “specific” to malignant tissues. Four decades or so later, Kano-Sueoka et al4 described that PEA acted as a growth factor of a rat mammary carcinoma cell line. In the last 10 years, six studies tested a PEA synthesized by Gilberto Chierice and co-workers (i.e., “synthetic” PEA) in \textit{in vitro} (cytotoxic effects in some cancer cell lines) and \textit{in vivo} (inhibition of xenograft tumor growth in rodent models) assays5,6,7,8,9,10. A tentative rationale for these experiments is the speculation that a massive presence of PEA in some malignant tumors (shown by Outhouse2,3) would indicate that PEA is overproduced to hamper neoplastic cell proliferation. In other words, an excess of PEA would be a kind of defense mechanism of the organism against uncontrolled cell proliferation and tumor growth. A corollary to this hypothesis would be that an additional supply of “synthetic” PEA (syn-PEA) could help patients to eliminate malignant cells or at least to control their proliferation. Nonetheless, except for the foregoing studies, no other scientific-based report supports - whether directly or indirectly - the notion that PEA would play a role in the process of carcinogenesis.

Reported anticancer effects of “synthetic” phosphoethanolamine

Gilberto Chierice and coworkers’ experiments showed that syn-PEA in the mM (10^-3) concentration range was toxic to some cancer cell lines (Table 1). Actually, these findings revealed that syn-PEA, however, have not undergone any targeted screening of compounds against cancer cell lines and uncharacterized tumor models, to a target-orientated screening of compounds with defined mechanisms of action. Inhibitors of enzymes playing a key role in the sustained proliferative activity, such as cyclin dependent kinases (CDK), for instance, are potentially new anticancer agents46. PEA and syn-PEA, however, have not undergone any targeted orientated screening of anticancer drugs.

As early as in 1936, Edgar Outhouse2,3 found substantial cell membranes (Figure 1).

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1 The hollow fiber assay was developed to bridge the gap between \textit{in vitro} cell line tests and \textit{in vivo} xenograft tumor assays in immunodeficient mice. Inert hollow fibers containing human tumor cell lines are transplanted into the peritoneal cavity or implanted under the skin of the host mice. The hollow fiber pores are small enough to retain the propagating cancer cells and large enough to allow the entry of potential anticancer compounds. After the \textit{in vivo} treatment hollow fibers are retrieved for analysis of the viable cell mass.20,21,22
Drug research and development process

Drug research and development (R&D) is a long, costly and complex multi-step process. In the first step (drug discovery), a number of natural or synthetic compounds are screened to select those with a pharmacological activity potentially useful in therapeutics. The subsequent step is a preclinical safety research (including a set of in vitro assays and in vivo animal tests) to decide whether the compound is reasonably safe for a first-in-man (phase 0) study and/or a phase 1 clinical trial. If investigators and regulators agreed upon that the compound would not expose healthy volunteers and/or patients to unacceptable risks of harm, a sequence of phase 1, 2 and 3 clinical trials (the final R&D step) takes place. Phase 3 trials provide the decisive evidence that a drug is effective and safe for the intended therapeutic use. R&D requires large capital expenditures (from hundreds of millions to billions of US dollars) and takes a long time (7-12 years). It also involves high attrition rates. (Figure 3)

![Diagram of Ethanolamine Kinase](http://visaemdebate.incqs.fiocruz.br/)

**Figure 1.** Phosphoethanolamine (PEA) also known as phosphorylethanolamine or O-phosphoethanolamine (CAS Nr 1071-23-4, C₂H₈NO₄P, Molecular mass = 141.063 g/mol) is an intermediate in the endogenous synthesis of phospholipids, components of cell membranes. PEA and ADP are the products of an ethanolamine kinase (EC 2.7.1.82)-catalyzed reaction the substrates of which are ATP (donor of phosphate group) and ethanolamine (alcohol group acceptor).

**Table 1.** Cytotoxicity of synthetic phosphoethanolamine (syn-PEA) to cancer and non-cancer cell lines.

<table>
<thead>
<tr>
<th>Study</th>
<th>Cell line</th>
<th>Assay</th>
<th>Cytopathic concentration range or IC₅₀</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferreira et al, 2012</td>
<td>B16-F10</td>
<td>MTT</td>
<td>12.5-100 mM</td>
<td>Mouse melanoma</td>
</tr>
<tr>
<td></td>
<td>MCF-7</td>
<td>MTT</td>
<td>1.82 mg/ml (16 mM)</td>
<td>Human breast cancer</td>
</tr>
<tr>
<td></td>
<td>Skmel-28</td>
<td>MTT</td>
<td>1.2 mg/ml (8.5mM)</td>
<td>Human melanoma</td>
</tr>
<tr>
<td></td>
<td>HeWo</td>
<td>MTT</td>
<td>2.4 mg/ml (17.1 mM)</td>
<td>Human melanoma</td>
</tr>
<tr>
<td></td>
<td>H292</td>
<td>MTT</td>
<td>1.32 mg/ml (9.4mM)</td>
<td>Human mucoid pulmonary carcinoma</td>
</tr>
<tr>
<td>Ferreira et al, 2012</td>
<td>Huvec-CRL1730</td>
<td>MTT</td>
<td>&gt;5 mg/ml (&gt;35.7mM)</td>
<td>Human umbilical vein endothelial cell</td>
</tr>
<tr>
<td></td>
<td>FN1</td>
<td>MTT</td>
<td>&gt;5 mg/ml (&gt;35.7mM)</td>
<td>Human normal fibroblasts</td>
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<tr>
<td></td>
<td>Lymphocyte</td>
<td>MTT</td>
<td>&gt;5 mg/ml (&gt;35.7mM)</td>
<td>Mouse lymph node</td>
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<tr>
<td></td>
<td>EAT</td>
<td>MTT</td>
<td>2.3 mg/ml (16.4 mM)</td>
<td>Mouse Erlich Ascites Tumor</td>
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<tr>
<td></td>
<td>B16-F10</td>
<td>MTT</td>
<td>1.4 mg/ml (10mM)</td>
<td>Mouse melanoma</td>
</tr>
<tr>
<td>Ferreira et al, 2013</td>
<td>KG-1</td>
<td>MTT</td>
<td>9 mM</td>
<td>Human myeloid leukemia</td>
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<tr>
<td></td>
<td>KG562</td>
<td>MTT</td>
<td>6 mM</td>
<td>Human erythroleukemia</td>
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<td></td>
<td>Jurkat</td>
<td>MTT</td>
<td>12 mM</td>
<td>Human T cell leukemia</td>
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<tr>
<td>Ferreira et al, 2013</td>
<td>MCF-7</td>
<td>MTT</td>
<td>20 mM</td>
<td>Human breast cancer</td>
</tr>
<tr>
<td></td>
<td>MCF-10A</td>
<td>MTT</td>
<td>100 mM</td>
<td>Human mammary epithelial cells</td>
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<tr>
<td>Ferreira et al, 2013</td>
<td>Renca</td>
<td>MTT</td>
<td>90 mM</td>
<td>Mouse renal carcinoma</td>
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<td></td>
<td>IRPTC</td>
<td>MTT</td>
<td>134 mM</td>
<td>Rat immortalized proximal tubule cells</td>
</tr>
<tr>
<td></td>
<td>Huvec CRL1730</td>
<td>MTT</td>
<td>73 mM</td>
<td>Human umbilical vein endothelial cell</td>
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<td>LOE-UFC, MCTI- 2016</td>
<td>HCT-116</td>
<td>MTT</td>
<td>25.9 mM</td>
<td>Human colorectal carcinoma</td>
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<tr>
<td></td>
<td>PC 3</td>
<td>MTT</td>
<td>19.7 mM</td>
<td>Human prostate adenocarcinoma</td>
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<td>SF-295</td>
<td>MTT</td>
<td>43.4 mM</td>
<td>Human glioblastoma tumor</td>
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<td>L929</td>
<td>MTT</td>
<td>8.6 mM</td>
<td>Mouse fibroblast</td>
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<tr>
<td></td>
<td>CMSP</td>
<td>MTT</td>
<td>42.4 mM</td>
<td>Human peripheral blood mononucleated cells - Primary culture</td>
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</table>

*Purity of PEA was >95%, then 1mM = 0.14 mg/ml. However, since syn-PEA is in fact a mixture containing a great amount of impurities, real molar concentrations of PEA in syn-PEA are possibly at least 30 to 40% lower than estimates listed above. LOE-UFC, MCTI - contracted laboratory.

3 Sunitinib. + Doxorubicin. Molar mass of PEA (C₇H₈NO₄P) is 141.063 g/mol. If purity of PEA were >95%, then 1mM = 0.14 mg/ml. However, since syn-PEA is in fact a mixture containing a great amount of impurities, real molar concentrations of PEA in syn-PEA are possibly at least 30 to 40% lower than estimates listed above. LOE-UFC, MCTI - contracted laboratory.
of 5–10,000 compounds that were initially screened for potential therapeutic activity only 250 prove to be sufficiently promising to undertake pre-clinical evaluation, and of those only 10 are eventually tested on humans. Moreover, only 9.6% of all drug candidates that start a phase 1 trial successfully pass a phase 3 trial and receive a marketing authorization (data for 2006–15) 25. Taking into account this extremely high attrition rate and the poor performance of syn-PEA on the initial screening tests, it seems fair to think that MCTI bet heavily on a substance fated to fail a properly tiered approach to drug R&D in which the decision to pass to the next step of tests is based on the outcome of previous step studies (the logic of which is to save money, time and resources in drug development).

**Brazilian government-sponsored development of syn-PEA anticancer pill**

Notwithstanding the weakness of scientific evidence for a syn-PEA-mediated anticancer activity, the MCTI allocated a substantial amount of taxpayers’ money to develop a syn-PEA-based oncologic drug 26,27,28. Amazingly, MCTI-sponsored studies on the safety of syn-PEA are in progress and a phase 1 test on humans is in preparation, while some basic studies are still looking for some pre-clinical evidence of anticancer activity 29,30,31. Apparently, there was an a priori decision to proceed R&D studies of syn-PEA up to the clinical phase irrespective of the previous nonclinical efficacy and safety investigation results.

In other words, MCTI-sponsored plan to develop syn-PEA as an oncologic medicine is a disarranged version of the conventional tiered approach to drug R&D in which the decision to pass to the next step of tests is based on the outcome of previous step studies (the logic of which is to save money, time and resources in drug development).

**Syn-PEA anticancer pill law**

A bill that authorizes production, prescription and consumption of syn-PEA as an oncologic drug passed the Congress, and the Brazilian president signed it (with no veto) into law (Federal Law 13269/2016) on April 13th, 2016 32. The syn-PEA law was challenged by a lawsuit (Direct Unconstitutional Action - ADIN) filed by the Brazilian Medical Association (AMB) and the full board of the Federal Supreme Court (STF) - by a 6 to 4 vote - suspended temporarily its effectiveness until a court final decision. The six STF ministers who voted for a temporary suspension of Law 13269/2016 explained the lack of clinical studies of syn-PEA in their declaration of vote 33.

**Ethical clearance for clinical trials of syn-PEA in Brazil**

Astonishingly, the Brazilian National Committee for Ethics in Research (CONEP) authorized (in April 19, 2016) a São Paulo State Secretary of Health-sponsored study of syn-PEA...
efficacy and safety in cancer patients\textsuperscript{31}. As aforementioned, Chierice and coworkers stated that syn-PEA has therapeutic usefulness in oncology is not supported by robust experimental evidence. Notwithstanding the fact that syn-PEA inventors have repeatedly said and disseminated in the lay press that cancer patients improved - or even cured - after taking the anticancer pill, they have not shown any clinical documentation (patient records) or published any case report to corroborate their statements.

Moreover, CONEP permitted the onset of clinical trials in the absence of a comprehensive (or even a minimum) preclinical evaluation of PEA safety. It is of note that CONEP neglected a possible harm to cancer patients. Data by Kano-Sueoka et al\textsuperscript{31}, suggested that PEA could be a tumor growth factor and one of the MCTI-contracted laboratories reported an apparent syn-PEA-caused enhancement of metastatic tumors in a rat xenograft tumor assay\textsuperscript{31}.

Approval of clinical studies of compounds for which sponsors provided no scientifically valid and convincing evidence of a potential therapeutic usefulness breaks a cornerstone rule of clinical investigation ethics. The CIOMS-WHO ethical guidance for research involving human subjects states explicitly that "...scientifically invalid research is unethical..."\textsuperscript{36} and that sponsors and investigators must ensure that "...studies involving human subjects...are based on adequate knowledge of the pertinent scientific literature"\textsuperscript{36}. Moreover, risks and benefits for the individual subject (i.e., the cancer patient) must be reasonably balanced and risks minimized (CIOMS guideline 8)\textsuperscript{36}. According to CIOMS-WHO guidance (comments to guideline 8) and to the Declaration of Helsinki (paragraph 11), "...clinical testing must be preceded by adequate laboratory or animal experimentation to demonstrate a reasonable probability of success without undue risk"\textsuperscript{36}. As far as the approved clinical trials of syn-PEA are concerned, a crucial question remains

\textsuperscript{3} National Cancer Institute - Brazil (INCa) and MCTI. Seminar on phosphoethanolamine (Seminário sobre fosfoetanolamina) held in May 17, 2016. The one-day INCa meeting was recorded and videos can be watched at: www.youtube.com/watch?v=q6Es2vn3Aw (morning session) and www.youtube.com/watch?v=fpCesJIRn (afternoon session).

\textsuperscript{4} CIOMS and WHO ethical guidelines state that (Guideline 1) "...because scientifically invalid research is unethical in that it exposes research subjects to risks without possible benefit. Investigators and sponsors must ensure that proposed studies involving human subjects conform to generally accepted scientific principles and are based on adequate knowledge of the pertinent scientific literature."\textsuperscript{36}. 

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\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{The US National Cancer Institute drug-screening paradigm. The Developmental Therapeutics Program (DTP) Anticancer Screening Paradigm is a tiered multi-step approach. Compounds are pre-selected based on their chemical structures and other data relevant for potential novel anticancer activity. In a second tier, preselected compounds undergo cellular assay (60 different human tumor cell lines) screening in two steps. The first cellular assay step is a 3-cell line / one concentration (dose) prescreen to select compounds with anti-proliferative activity to be further tested against the full 60-tumor cell line panel over 5 concentrations (doses). After a preliminary review of data, novel compounds with identified growth inhibition or killing of tumor cell lines activity undergo acute toxicity testing (to determine maximum tolerated doses) and the hollow fiber assay on rodents. Based on a review of available data, investigators then decide whether a compound should further undergo rodent xenograft tumor assays. As shown in the diagram (adapted from DTP flow chart and information available on https://dtp.cancer.gov/discovery_development/default.htm), compounds undergo "No Further Testing" (NFT) if they fail to pass a previous tier testing.}
\end{figure}
unanswered: Based on available preclinical scientific data, what are the prospects of benefits for the individual subject (cancer patient)? Needless to say it again: syn-PEA (and PEA) did not undergo a properly conducted screening for anti-cancer activity, and initial tests (cytotoxicity and xenograft assays) yielded disappointing results which according to US NCI-screening paradigm would not support further testing (Tables 1 and 2, Figure 2).

The potential risks of syn-PEA to clinical research subjects were not adequately investigated either. As stated by ICH guidelines (e.g. ICH guidelines for non-clinical safety studies to conduct clinical trials), “nonclinical safety studies ….. should be adequate to characterise potential adverse effects that might occur under the conditions of the clinical trial to be supported”. Moreover, nonclinical safety evaluation must include repeated-dose studies in two species (one non-rodent) the duration of which should be at least equivalent to that of the clinical trial to be supported (e.g., to support a 6-month clinical trial, durations of nonclinical repeated dose assays must be 6-month or longer). It is of note that CONEP approved the onset of São Paulo State Secretary of Health-sponsored clinical studies of syn-PEA before the results of the MCTI-contracted preclinical studies (i.e., cytotoxicity assays, acute toxicity and 30-day repeated dose study in rodents) were available. At any rate, the limited set of preclinical studies contracted by MCTI is far from being sufficient to support a clinical study of syn-PEA in cancer patients.

The current approval of syn-PEA clinical study by CONEP without an adequate preclinical evidence of anticancer efficacy and safety may suggest that Brazilian ethical review system allows desperately ill patients (a particularly vulnerable group of people) to volunteer to be “guinea pigs” for poorly tested or even untested drugs. This was certainly the most worrisome consequence of syn-PEA sad story: it made a case law for further approval of

\[\text{Figure 3. Drug Research and Development (R&D) process. R&D begins with a screening for pharmacological effect of therapeutic interest (compounds generally obtained by prospecting natural products or chemical synthesis) and preclinical toxicity evaluation through in silico, in vitro, and/or short-term in vivo assays. A review of data on genotoxic potential, pharmacokinetics, safety pharmacology and repeated dose toxicity in at least two species (one non-rodent) and all relevant information available (and clinical trial protocols) precedes the beginning of the clinical research and development phase. Clinical R&D is a three-step investigation process involving a sequence of phase 1 (including a small number of healthy volunteers or patients, focus placed on safety), phase 2 (controlled trial involving a larger number of patients diagnosed with the medical condition the compound is intended to treat, focus placed on safety and efficacy; it is a pilot dose-ranging trial), and the final phase 3 study. Phase 3 trial is a decisive research step to demonstrate drug efficacy and safety to treat the disease of interest. The clinical trial should be controlled, double-blinded, randomized, statistically robust (adequate number of subjects enrolled), and to assess valid clinical outcomes of efficacy. Nonclinical long-term safety studies (reproductive toxicity, carcinogenic potential, long-term repeated dose toxicity) are performed in parallel with the clinical phase. A comprehensive review of all R&D data with emphasis on phase 3 trial results precedes a drug marketing authorization. Post-marketing surveillance is necessary to reveal drug problems of effectiveness under real (marketing) conditions of use and rare adverse events that escape detection in phase 3 trials. Attrition rate of R&D is extremely high and of 5-10,000 compounds that apparently have a pharmacological activity of therapeutic interest only 1 eventually succeeds in achieving a marketing approval. In Brazil, the syn-PEA law (Law 13269/2016) authorizing the manufacture, prescription and dispensing of this putative anticancer pill is a radical shortcut to this normally lengthy, costly and highly selective way to approve a drug for marketing.} \]
clinical investigations of drug candidates insufficiently evaluated by preclinical studies.

Concluding remarks

It is very unlikely that syn-PEA or PEA-based medicines will eventually play a role in cancer treatment. The syn-PEA anticancer pill story, however, is unique in several aspects. Manufacture, prescription, dispensing and consumption of syn-PEA was authorized by a Federal Law (the effectiveness of which was suspended by a STF temporary decision). The Congress overwhelmingly passed the syn-PEA bill and the president signed it into law despite the fact that the Anvisa, the Brazilian Society for Advancement of Science (SBPC) and the country’s physician association (AMB) strongly recommended Congress members and the president not to do it\(^5\). The MCTI also allocated substantial public funds to support R&D of an unlikely new anticancer drug. Furthermore, CONEP, the highest-level and most influential committee on human research ethics in Brazil, authorized the onset of syn-PEA trials in cancer patients in the absence of convincing evidence of anticancer activity and without an adequate and comprehensive set of preclinical safety data. Damages to the country’s international reputation regarding national drug regulation policies and human research ethical standards are unavoidable. The anticancer pill story, however, taught us an important lesson: the strength of popular beliefs in unsubstantiated allegations, whenever unethical politicians capture and endorse them, should not be underestimated. Incumbent politicians’ ambitions, depending on a well-orchestrated lobby and other circumstances, eventually take precedence over general public interests, ethical principles and scientifically based decisions on drug regulatory affairs, thereby misleading decisions on scientific research support and on the approval of clinical study protocols.

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