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Original Article

A comparative review of the potential role of adenovirus and Herpes Simplex Virus in the treatment of advanced squamous cell carcinoma of the head and neck

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Abstract

The unsatisfactory outcome of patients who receive intensive multimodality treatment for advanced squamous cell carcinoma of the head and neck (SCCHN) has motivated investigators to seek novel treatments to improve survival. Advances in molecular biology has led to the development of cancer gene therapy (CGT) and revived interest in viral vectors as a mechanism. SCCHN is an ideal model for CGT as disease remains locoregional and is amenable to injection of viruses. Adenovirus and Herpes Simplex Virus Type-1 (HSV) are the most studied Oncolytic Viruses (OVs). Both viruses have been shown to select and replicate in tumour cells and demonstrate anti-tumour effect in laboratory studies and clinical trials. Toxicity from OVs is minor and manageable. Different adenoviral mutants have been investigated with mixed responses. One vector, H101, has now been licensed after showing significant tumour regression in conjunction with chemotherapy. HSV has a larger capacity to carry genetic material and with the addition of the granulocyte–macrophage colony–stimulating factor, has the potential to stimulate an immune response systemically and at the site of disease. OVs are limited by the distribution of virus beyond injection site and by pre-existing or rapidly established immune response. Phase III studies are required.

Keywords

Adenovirus; Cancer gene therapy; Head & neck cancer; Herpes simplex virus

INTRODUCTION

Squamous cell carcinoma (SCC) is the most frequent type of cancer observed in the head and neck. It ranks as the sixth most common cancer globally, and for patients presenting with the disease 60% will be found to have locally advanced disease.[1–3] Advanced SCC of the head and neck (SCCHN) has a poor prognosis. Without treatment a patient has a 50% chance of death within 4 months and even with the combined modality of radiotherapy, chemotherapy and/or surgery the 5-year survival rate can be <50%.[4,5] The main cause of death for SCCHN patients is due to uncontrolled local disease.[6] This has necessitated the development of aggressive combined treatment regimes of radiotherapy and chemotherapy.[6]

Those patients who do achieve cure are prone to severe acute toxicities and a potential multitude of debilitating and chronic late conditions such as radionecrosis, xerostomia, fibrosis and toxicity to the renal system, the gastro-intestinal tract

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and bone marrow.[7] Salvage surgery is often employed for patients with recurrent disease but can result in deformity and can have adverse effects on speech, swallowing and global quality of life.[7] Palliation is fraught with difficulty, <40% of patients respond and any benefit that is seen only lasts 6–9 months.[2,8]

This combination of unsatisfactory treatment outcomes, coupled with significant treatment toxicities and limited palliative options has led researchers to seek novel therapies to improve the prognosis for SCCHN patients. One such area is that of cancer gene therapy (CGT). CGT is being researched as a treatment to work additively or as a single modality for the treatment of SCCHN.

BACKGROUND

CGT theory is to introduce genetic material to tumours in order to cause a tumouricidal effect; these effects can be direct, i.e. at the individual cellular level or indirect, i.e. stimulating adjacent uninvolved tissue to create an immune response.[9] SCCHN has been identified by researchers as being amenable to the development of CGT as the disease is mostly restricted to the locoregional area and it is in close proximity to the body's surface and is therefore easily accessible to injection.[9]

Viruses are a popular form of vectors for CGT and are a subject of a great deal of research.[10] The term "vectors" indicates a mechanism or delivery system by which genetic material is transferred into cells.[10] From the earliest part of the 20th century, it was observed that certain cancers went into remission after the patient had suffered an episode of viral infection. This led to research being conducted on a variety of solid tumours with viruses by the 1950s. However, work ceased due to intolerable toxicities in patients.[11]

Advances in molecular biology have led to the renewed interest in viruses for the treatment of cancer and these are known as Oncolytic Viruses (OVs). In 2004, there were 88 clinical trials underway and 74% were focussed on cancer.[12] Viruses are adept at infecting and killing a broad range of human cells. They have the ability to alter the genetic composition of host cells and it is of notable importance that viruses can induce changes in cells similar to those that occur during cancer development itself.[13] This is one of the means by which viruses can target cancer cells.[13]

OVs must have certain qualities: the ability to efficiently infect, replicate within and ultimately lyse cancers cells; the ability to transduce both proliferating and non-proliferating cells; and the causation of virus-related illness should be limited to minor diseases.[13] The two viruses that have received the most attention as OVs are Adenovirus and Herpes Simplex Virus type-1 (HSV1).[11]

This paper will critically examine and compare these two viruses for the treatment of SCCHN.

ADENOVIRUS

Adenoviruses are a common cause of upper respiratory tract infections and over 50 serotypes have been observed.[14] Adenovirus is a non-enveloped virus with a linear double-stranded genome[14] (see Appendix I). Adenovirus is the most popular of currently studied OVs because it can replicate in dividing and non-dividing cells, it does not alter the host cells genetic sequence and it has the capacity to carry sizeable portions of exogenous DNA.[12,14] Adenovirus targets and gains entry to cells via the coxsackie-adenovirus receptor.[12] The use of adenovirus has been linked to one death, associated with intra-hepatic viral treatment for a young man with a metabolic disorder, which caused massive inflammation.[12] This led to the removal of all unnecessary viral genes.[12]

A number of genetically engineered mutant adenoviruses have been created, which focus on the tumour suppressor protein p53 for cancer cell targeting.[10] In SCCHN between 26 and 77% of tumour cells have p53 mutation.[10,15] Other replicating adenoviruses make

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use of the proteins E1A and E1B to influence cell-cycle progression through to the S phase in order to prevent apoptosis; this allows viral replication to take place.[10]

Adenoviruses with p53 mutation have been reported to demonstrate high tumour suppression in cell lines of SCCHN and using the same virus in vivo with nude mice prevented the establishment of tumours after subcutaneous implantation.[16] These results were taken from laboratory controlled conditions but were encouraging enough that other research was stimulated.

A further study investigated a mutant E1A adenovirus that was tested against a variety of human cancer cell lines in vitro and with tumour bearing mice in vivo.[17] This mutant demonstrated selective replication in both dividing and non-dividing cells and a good response in vivo.[17] This particular virus showed an ability to infect cells regardless of the p53 status. This is a distinct advantage as not all cancer cells have the mutation and this would inherently increase tumour selectivity. The efficacy of this virus was statistically significant but disappointingly no other researchers have studied it.[17]

Another mutant variant, this time with both E1A and E1B mutations was investigated and was found to have a dramatic increase in transfection rates in vivo for the mutant virus.[18] In this study, there was high anti-tumour efficacy in vivo but the authors reported differing volumes of the tumours from the nude mice so exact responses were difficult to quantify. The exact mechanism by which improvements in efficacy in this study took place were not clear to the authors.[18]

For clinical trials the most prominently studied adenovirus mutant for SCCHN has been ONYX-015.[15] This virus has the E1B 55-kDa gene removed. This gene is responsible for the binding and inactivation of p53 and so without it the virus cannot replicate in the normal cells that have a functional p53 protein.[8,15] Two studies were undertaken to assess the role of ONYX-015 for the treatment of patients with recurrent SCCHN.[8,15]

In the first of these, 20 patients were involved who had either lymph node involvement or skin metastases. The researchers then measured the tumours and divided them into sections. These sections were of 1 cm^2 sizes and had either virus or a control fluid injected intratumourally.[15] There was a great deal of difference in the size of tumours investigated in this study, ranging from 2.2 to 20 cm.[15] The intention of the study was to allow up to five treatments with the virus if there was either a response, no evidence of progressive disease and if no dose limiting toxicity (DLT) was seen.[15] There were no statistically significant clinical responses seen.[15] All patients either progressed at the site of injection or developed new disease. Though these results were disappointing there was evidence of anti-tumour activity in the injected tumours and the study demonstrated the safety and feasibility of using ONYX-015, both in terms of tolerability of side effects and also in that there was no evidence of virus shedding in blood or at the injected site.[15] Overall 23% of patients had some level of measurable response.[15] The inclusion of tumours of varying size was problematic. This made it more difficult to assess and compare individual tumour response.[15]

The second trial also made use of the intratumoural injection of ONYX-015 but in this trial, patients also received cisplatin and 5-Fluorouracil.[8] This gave the researchers the opportunity to compare the effects of the virus combined with chemotherapy compared with the chemotherapy alone. The largest or most symptomatic tumours were injected while leaving other tumours un-injected as control.[8] The treatment was well tolerated, no viral replication was observed in normal tissue and the individual toxicity of virus and chemotherapy was not worsened by the combination of both modalities.[8] This study demonstrated a tumour mass reduction of >50% in 63% of the patients.[8] Overall tumour response rate and complete response rate were superior in the injected tumours. This result, coupled with the much greater reduction in tumour mass, indicated a selective and additive benefit of the virus when combined with chemotherapy. These were exciting results as the development of

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chemotherapy resistance by tumours is a major obstacle to successful treatment.[8] These results were encouraging but impact on survival is un-measurable without phase III trials. This result incorporated heterogeneous tumour sites and sizes, the sample size was small (incorporating 37 patients) and it can be argued that, once again, the maximum tolerated dose of the virus was not reached.

This observed benefit was given greater credence when the result of phase III randomised trial using H101 (an E1B deleted mutant) for the treatment of SCCHN was published.[19] In this study, patients were randomly assigned to receive cisplatin and 5-Fluorouracil with or without H101 injections.[19] The responses were statistically significant. The overall response rate from H101 plus chemotherapy as compared to chemotherapy alone was 78.8% vs 39.6%.[19] The result of this trial has led to a licence being granted for routine use of H101.[19]

A recently published paper undertook research to further the enquiry into the relationship between chemotherapy and adenoviral mutants but also sought to determine whether the selection, or deletion, of certain genes made an impact on this relationship.[20] This study incorporated both in vitro and in vivo examination and demonstrated that the efficacy of the virus was increased when given concurrently with cisplatin and paclitaxel.[20] This is partly due to the ability of chemotherapy to influence immune cells. The role of the E1A gene was found to be essential in making infected cells sensitive to chemotherapy and that even mutants with E3B deletions, which are prone to swift clearance by the immune system, had greater anti-tumour activity when combined with chemotherapy.[20] The authors did comment that in terms of in vitro results the beneficial combination was heavily dependant on the individual cell line (none of which was from a human SCCHN tumour) and the exact level of amount of drug and virus.[20] The paper also poses two problematic areas: first, how important is this ratio in determining the amount of additive benefit seen and, second, considering the changeable and complex nature of the tumour microenvironment, there is a question whether this is going to realistically achievable in treated patients. While this study indicates some potentially useful research options there is a lack of corroborative evidence at present.

In addition to the benefit observed with chemotherapy, there has also been a link established with OVs and radiotherapy.[21] Studies have shown that ONYX-015 in concurrent treatment with radiotherapy yields an interactive benefit with in vivo studies in a variety of tumours.[21] Researchers have indicated that the interaction of adenoviral mutants and radiotherapy is multifaceted and that lower doses of viruses were needed to produce similar anti-tumour results as seen with chemotherapy.[21]

It has been seen that mutants of adenovirus have been able to successfully select and replicate in SCCHN cells and they have shown differing levels of tumouricidal effects in vitro, in vivo and in the treatment of patients with recurrent disease. Results have varied and in those studies in which notable responses were seen, the method by which they have come about is not fully understood. This is partly due to study design, differences in viral dosage and also due to the complex factors that influence adenoviral efficacy.[22] The differing roles of genes in the adenoviral mutants can have direct cytotoxic effects, their deletion can also slow down apoptosis and genes can sometimes work antagonistically.[22] The composition of the adenoviral mutants are complex and the ideal not yet reached. This is even more critical for adenoviral mutants as it has a limited capacity for delivery of genetic material as compared to other viruses.[22]

HERPES SIMPLEX VIRUS

Herpes Simplex Virus is an enveloped double-stranded DNA virus[23] (see Appendix I). It causes cold sores, herpes and rarely, encephalitis; it has a natural affinity for neural tissues and can lie dormant, causing disease at a date long past infection.[23,24] There are two serotypes HSV-1 and HSV-2.[24] The virus replicates efficiently in the nuclei of infected cells, and can

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produce thousands of progeny within a 24-h period.[23] Another advantageous feature of HSV is that it can carry a large amount of DNA due to the fact that the virus can still function with almost the whole genome repackaged with different genetic material.[24] HSV-1 has been the serotype investigated as a viral vector. HSV-1 can be engineered to preferentially target tumour cells. This is achieved by the deletion of certain proteins (ICP34.5) that are necessary for viral replication. These required proteins are over-expressed in tumour cells and thus the virus is able to use these to complement the loss of ICP34.5.[10] It is also worth commenting that the vast majority of all the HSV-1 vectors that have been investigated share this type of genetic attenuation.[25]

The first HSV-1 mutant employed in research was a vector coded 'dlsptk'. However, significant safety issues in terms of neurotoxicity were raised and this led to the deletion of the genes responsible.[24] A different mutant, NV1020, was investigated as part of an in vitro and in vivo study.[1] The in vitro component examined the effect of the virus on five different SCCHN lines. The virus was able to replicate efficiently in four of the cell lines and in vivo tumours originating from tongue lesions proved most sensitive, showing complete regression within 15 days.[1] It must be added that there was a wide range of response from the different tumour sites.[1] Despite that, this study focussed on human SCCHN and demonstrated both in vitro and in vivo anti-tumour efficacy. The virus was well tolerated and the authors suggested that this mutant might be of use in patients with recurrent disease.[1]

In a pilot study of two elderly patients, the vector HF10 was examined in the treatment of recurrent SCCHN.[26] Both these patients had received standard local treatment and had recurred, one with skin nodules, the other with lymphatic deposits.[26] Both patients received three injections intra-tumourally over 3 days, were monitored for viral toxicity. Injected disease was then measured and excised at day 13 for the first patient and day 15 for the second. It was found that the virus infected and replicated efficiently in tumour cells and though there was no significant regression in disease there was measurable cell death caused by the virus.[26] The authors comment that this vector was 10,000-fold less potent than the wild type HSV-1.[26] No explanation is given as to why the authors chose to investigate such an attenuated mutant, or why they chose to excise the injected disease at different times, allowing one tumour a further 2 days for viral activity. This was small, even for a pilot study, and there was no differentiation in the discussion between the responses from the two different tumours, an indicative study at best.

A more recent study examined the role of a different viral vector, Oncovex$^{GM\text{-}CSF}$ (Oncovex). This vector includes GM-CSF, the granulocyte–macrophage colony–stimulating factor.[27] Oncovex was engineered to be more efficacious as it has an additional genetic deletion in the viral ICP47 gene. This deletion promotes antigen presentation in SCCHN cells and the inclusion of GM-CSF promotes the immune system for the production of specific dendritic cells.[27] Oncovex demonstrated significant tumouricidal effects in laboratory studies. Patients with recurrences from a number of different tumour sites were injected with different levels of Oncovex. There were no DLTs observed in this study and inflammation at injection site plus flu-like symptoms were the most common problems reported.[27] Though no objective responses were seen, the study's main end points were to assess the safety and replication of Oncovex and these were demonstrated. There was evidence of GM-CSF expression and viral-linked tumour necrosis.[27] The differing levels of viral dosage was linked not only to dose escalation but also to individual tumour mass and this adds slight confusion as to whether the tumours received the same equivalent dose of Oncovex.

HSV-1 also demonstrates a synergism with chemotherapy and in respect of combination with radiotherapy, it can produce more complete responses in vivo. These beneficial effects are not isolated to individual mutant types.[21,28]

Previously in this author's current workplace, a Phase I/II exploratory study into the safety

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and biological activity of Oncovex in combination with radiotherapy and cisplatin for the treatment of SCCHN was carried out. This author took no active part in any research[29] (see Appendix II for overview). The primary objective was to assess safety and the secondary objective to assess any biological activity by radiological and immunological testing. Seventeen patients were recruited.[29] Patients had stage III or IV disease with at least one nodal metastasis. If patients had more than one involved node then one would be left uninjected as a control. This is a single arm study with three differing dose levels of Oncovex.[29] Patients were scheduled for neck dissection after completion of all treatment. Early indications from this study have been positive; no DLTs were observed, only two patients demonstrated evidence of virus shedding from the injection site and there were pathological complete remissions shown from the neck dissections in the majority of patients[29] (see appendix II for nodal response before dissection). Personal communication from the principal investigator indicates that the addition of Oncovex injections had no significant adverse impact on the management of these patients whilst on treatment.[29] From the radiographer's perspective there was little negative impact. The scheduling of patients appointment time had to be timed to co-ordinate the injection and chemotherapy administration but this was organised with ease and with minor impact on service delivery. A safety precaution of not allowing pregnant staff to treat patients was not problematic. This precaution was put in place as the researchers did not know if the modified virus could be spread.

DISCUSSION

Both the viruses in this comparative review have shown themselves competent as OVs. From Table 1 we can compare the differences between Adenovirus and HSV that are small but potentially significant. There has been a greater variety in the genes selected and deleted for adenoviruses and these have sometimes been counter-productive.[22] Work on HSV-1 has predominantly used the same genetic attenuation. HSV-1 has the greater viral capacity to carry genetic material and this could be a particular advantage as CGT strategies develop. In addition, Oncovex has the theoretical potential to stimulate a local and general immune response.[12] If this potential is realised it could have a significantly beneficial effect on the management of SCCHN patients both for local and systemic disease.

While both OVs have proven themselves to demonstrate anti-tumour activity in laboratory studies, only an adenoviral mutant has shown any kind of significant clinical response in a published trial.[19] This situation may change in respect of Oncovex if published data mirrors the unpublished preliminary clinical effects which show some potentially significant clinical results.[29] Both OVs have now been established as feasible and safe with viral use causing only

Table 1. *Overview of adenovirus and HSV-1 as Oncolytic Viruses*

	Adenovirus	**Herpes Simplex-1**
Gene deletions in vector	Variable	Consistent
Selectively targets SCCHN cells	Yes	Yes
Ability to carry genetic material	Moderate	Large
Able to infect & replicate	Yes	Yes
Virus active in dividing & non dividing cells	Yes	Yes
Evidence of antitumour activity in vitro & in vivo	Variety of responses	Variety of responses
Feasible & safe use	Yes	Yes
Response in range of SCCHN tumours	Limited	Yes
Able to induce a general immune response	No	Yes
Significant response from PI/II trials	Limited	Limited
Significant response from PIII trials	Yes	No trials as yet

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minor toxicities. We have also seen that OV use is most effective when combined with chemotherapy and radiotherapy.

General factors have limited the efficacy of both OVs and include the limitation of virus distribution beyond injection site, the effects of pre-existing or swiftly established immune response to the virus and the fact that OVs have been used as a single modality.

In conclusion neither virus can be described as being overall superior as a CGT vector. What is clear is that both OVs warrant further, more refined examination. Researchers need to assess both viruses in phase III studies to observe any impact on disease-specific and overall survival.

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APPENDIX I

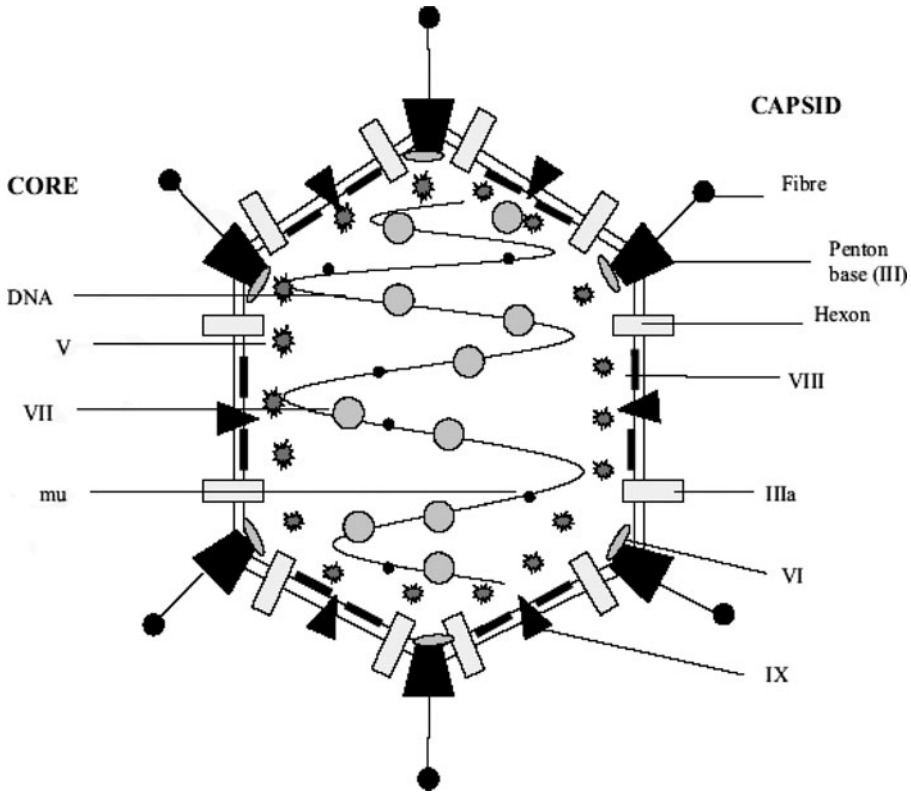

Figure 1. Structure of adenoviral capsid.

Taken from: Harrington K, Hardev P, Vile R (ed). Viral Therapy of Cancer. Wiley & Sons Ltd, 2008, 1–17.

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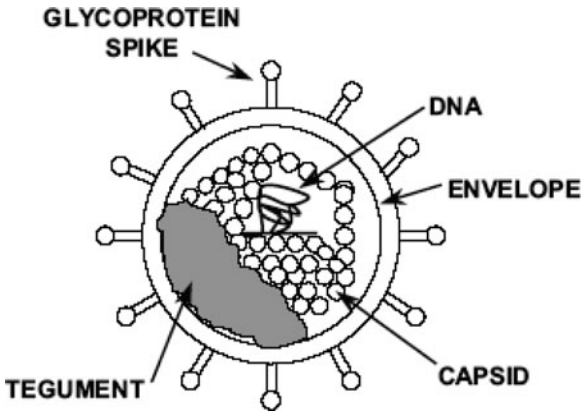


Herpes simples virus type-1

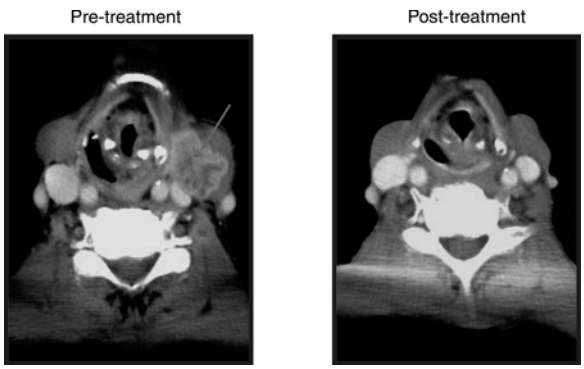
Taken from: Harrington K, Hardev P, Vile R (ed). Viral Therapy of Cancer. Wiley & Sons Ltd, 2008, 19–53.

APPENDIX II

Phase I Dose Escalation Trial of $HSV^{GM\text{-}CSF}$ Plus Chemoradiotherapy in Stage III/IV H&N Cancer

- Stage III/IV SCCHN (N1-N3 disease)
- Radical RT 70 Gy in 35 fractions
- Cisplatin 100 mg/m^2 day 1, 22, and 43
- $HSV^{GM\text{-}CSF}$ i.t days 1, 22, 43, 64
- Dose escalation (cohort n = 4)
  - 10^6, 10^6, 10^6, 10^6
  - 10^6, 10^7, 10^7, 10^7
  - 10^6, 10^8, 10^8, 10^8
- Neck dissection at 10-12 weeks

Trial overview & CT image below shared with permission of Personal Communication with Principal investigator 17.04.08



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