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Transgenic avian-derived recombinant human interferon-alpha2b (AVI-005) in healthy subjects: an open-label, single-dose, controlled study.

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Short Title
AVI-005 (IFN-α2b) in Healthy Volunteers

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ABSTRACT

Background/Aims

This study characterized the safety and pharmacologic properties of AVI-005, a novel glycosylated recombinant human interferon-α2b produced from the egg whites of chickens transfected with human cDNA.

Methods

Sixteen healthy volunteers received single rising doses of AVI-005. A subcutaneous dose of 0.5, 1.66 or 5 million international units (MIU) was administered. A randomized parallel comparator group of 10 subjects received 5 MIU unglycosylated IFN-α2b (Intron A). The pharmacokinetic parameters $t_{1/2}$, T_{max}, C_{max}, $AUC_{0-24\ hr}$, V_d, and clearance were compared between AVI-005 and unglycosylated IFN-α2b.

Results

At equipotent doses, AVI-005 generated a larger AUC_{0-24hr} than the control interferon. Pharmacodynamic markers of neopterin and β2 microglobulin were similar between the two treatments. These markers demonstrated dose dependent increases to AVI-005. Pharmacodynamic measures of mRNA expression of PKR and 2'5' oligoadenylate synthetase demonstrated a response to treatment with AVI-005. Adverse events were qualitatively and quantitatively similar in both groups.

Conclusion

AVI-005 demonstrates biologic activity and pharmacokinetic properties in humans that support further development.

INTRODUCTION

The interferons (IFNs) are immunomodulatory cytokines with antiviral and antitumor properties. (1). IFN-α is produced by leucocytes in response to viral infections. (2) Interferon -α2b (IFN-α2b) is an alpha interferon subtype used in the treatment of chronic hepatitis B and C, malignant melanoma, follicular (non-Hodgkin's) lymphoma, AIDS-related Kaposi's sarcoma, condylomata acuminata, and hairy cell leukemia. In humans, the native form of IFN-α2b consists of a single polypeptide chain of 165 amino acids with O-linked glycosylation at residue threonine-106. There are presently two FDA-approved recombinant IFN-α2b products, Intron A, and PEG-Intron (Schering-Plough, Kenilworth, NJ). Intron A is bacterial-derived non-glycosylated protein with an amino acid sequence identical to the natural IFN-α2b. PEG-Intron is comprised of the same underlying polypeptide chain linked to polyethylene glycol (PEG). This synthetic glycosylation provides improved efficacy with once-weekly administration. (3)

The current standard of care for the treatment of hepatitis C virus (HCV) infections is an interferon plus ribavirin. (4) Approximately 55% of patients with the most prevalent form of HCV do not respond to currently available interferon therapeutics. According to the World Health Organization, 200 million people, or 3% of the world's population, are infected with HCV with 170 million chronic carriers globally at risk of developing liver cirrhosis and hepatocellular carcinoma. (5) The Third National Health and Nutrition Examination Survey (NHANES III) found that 1.8 percent of Americans (3.9 million) have been infected with HCV. (6) An additional 400 million people worldwide are chronically infected with hepatitis B virus (HBV). (7) Thirty percent of HBV patients who tolerate interferon therapy have successful response. (8) Despite proven efficacy, the high cost of producing IFN-α2b by present methods places this treatment out of reach of the large majority of patients worldwide who suffer from these interferon-remediable infections.

AVI-005 is a transgenic human IFN-α2b produced by purification of chicken egg whites from hens transfected with human IFN-α2b cDNA. The AVI-005 manufacturing process involves 1) construction of a vector carrying the human IFN-α2b transgene, 2) insertion of IFN-α2b transgene into the chicken genome using embryo transduction technique, 3) screening of hatched birds to identify birds carrying the human transgene 4) breeding and characterization of transgenic birds to identify a founder (G1), 5) characterization of the G1 founder and its subsequent generations to confirm stable transmission of the IFN-α2b transgene through multiple generations and 6) harvesting and purification of the AVI-005 protein from the egg white. An advantage of this process is the ability to generate relatively large amounts of transgenic protein, which may translate into reduced production costs of recombinant human IFN-α2b. The amino acid structure of AVI-005 matches that of the native human IFN α2b. AVI-005 differs from the existing commercially available IFN-α2b products in that the threonine residue at position 106 is glycosylated with carbohydrate chains making it chemically identical to endogenous human IFN-α2b.

The goal of this first-in-human study was to characterize the safety, tolerability, pharmacokinetics and pharmacodynamics of AVI-005 in healthy human volunteers upon intramuscular administration of rising single doses, and to assess similarity to the existing commercially available IFN α2b product Intron A, henceforth described as “control interferon”.

PATIENTS AND METHODS

Subjects

Twenty eight healthy volunteers were enrolled in this study. Informed consent was obtained from each subject prior to any study procedures. The study protocol conforms to the ethical guidelines of the Declaration of Helsinki as reflected in approval by the Institutional Review Board of Thomas Jefferson University. Subjects

were deemed to be in good health on the basis of medical history, physical examination, laboratory values, and electrocardiograms (ECG). No subjects used prescription or over-the counter medications 2 weeks prior to study start. The mean age of the subjects was 31 years (range 19-49). Twenty-two (79%) of the subjects were male. Seventeen (61%) subjects were white, 8 (28%) were black and 3 (11%) were Hispanic.

Study Design

This was an open label, single rising dose study of intramuscular AVI-005 in healthy subjects. At the highest dose level, subjects were randomized to either AVI-005 or an equivalent dose of control interferon in a parallel design. The treatment groups were as follows: 1) 0.50 million units equivalent of AVI-005 (4 subjects), 2) 1.66 million units equivalent of AVI-005 (4 subjects), and 3) 5.00 million units equivalent of AVI-005 or control interferon (10 subjects in each treatment arm). The selection of the dose was based on preclinical data in primates employing AVI-005 doses of 0.13, 0.9, and 4.7 μg/kg which correspond, respectively, to 0.3, 2.2, and 11 times the human doses used in the current trial. The equivalency of AVI-005 doses to control interferon doses was determined by a bioactivity assay calibrated with one used for control interferon using a defined reference standard.

Each subject received a thorough physical exam, clinical laboratory safety assessment (hematology, electrolytes, liver function tests, and urinalysis) and ECG during screening. Female subjects had negative serum pregnancy test during screening and a negative urine pregnancy test the morning of study drug administration. Study subjects received a single intramuscular injection in the left buttock followed by pharmacokinetic and pharmacodynamic sampling. ECG's were performed at screening, within 1 hour of dosing, at 8 hours, and 3 and 30 days post study drug administration. Injection sites were observed for signs of irritation or inflammation.

Pharmacokinetics

Blood for AVI-005 pharmacokinetics was drawn at baseline and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48, 72, 96, 120 and 144 hours after drug administration. Subjects receiving control interferon had blood drawn for pharmacokinetics at predose; and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours after drug administration. Serum levels of AVI-005 and control interferon were determined using an interferon alpha specific ELISA. A 96-well plate was coated with an interferon alpha specific monoclonal antibody (Chemicon, Temcula, CA). Following blocking of the non-coated surface area with bovine serum albumin (Sigma, St. Louis, MO), the appropriate sample or standard was added. Captured interferon-α2b was detected with a secondary polyclonal anti-IFN alpha antibody which was indicated by a third alkaline phosphatase-conjugated antibody. The amount of IFN-α2b in a tested sample was indicated by the increase in the optical density at 450 nm after addition of alkaline phosphatase substrate. Concentration was calculated by interpolation using a standard curve generated with either AVI-005 internal standard or control interferon.

Pharmacodynamics

Pharmacodynamic analysis consisted of serial determination of serum analytes neopterin and β2 microglobulin and of messenger RNA (mRNA) expression of the interferon-inducible protein kinase (PKR) and 2'5' oligoadenylate synthetase (OAS). Neopterin, a pyrazinopyrinidine compound, is produced by macrophages after induction by interferons and serves as a marker of cellular immune system activation. (9) β2 microglobulin is a protein found on the surface of many cells, including white blood cells. Increased production of white blood cells, as seen with interferon administration, causes β2 microglobulin levels in the blood to increase. PKR is an interferon-inducible serine/threonine protein that is part of the innate immune response and inhibits viral replication. Its mechanism of action involves RNA-dependent autophosphorylation, leading to inhibition of translation. (10, 11) OAS is one of many genes encoding enzymes with antiviral activities. It is

important for antiviral response, and its activity is required by cells to activate the endonuclease RNase L, which degrades RNA. (12).

Blood for measurement of neopterin and β2 microglobulin was collected at predose, 24, 48, 72, 96, 120, and 144 hours post dose. Five subjects in the control interferon group had these analytes measured only at predose, 24, 48, and 144 hours. Blood for PKR and OAS mRNA was collected using a protease inhibitor RNALater (Ambion, Inc., Austin, TX) or Paxgene collection tubes (Qiagen, Valencia, CA) at predose, 8, 12, 24, 48, and 72 hours post study drug administration. Five subjects who received the control interferon did not have a 72-hour sample for PKR or OAS.

Pharmacokinetic Data Analysis

Pharmacokinetic analysis was performed using WinNonLin Enterprise version 4.1 (Pharsight Inc., Mountain View, CA). A non-compartmental approach was employed. Analysis of pharmacokinetics was performed out to 24 hours. Area under the curve (AUC) was determined using the linear trapezoidal rule. A terminal elimination constant was determined if judged appropriate by visual examination of the terminal time points. In cases where a terminal elimination could not be determined, only $AUC_{0\text{-}24\ hr}$, maximum concentration (C_{max}), and time to maximum concentration (T_{max}) were generated. For those in whom a reliable terminal elimination constant could be determined, half life, clearance, and volume of distribution were determined. Analysis of data from subjects receiving AVI-005 0.5 MIU was not performed due to concentrations below the limit of quantification.

Statistical Methods

Pharmacokinetics

AUC and C_{max} values were natural log-transformed. Means, standard deviations, minimums, maximums, and medians were calculated for each dose level and treatment group. Arithmetic means were back-transformed (by taking anti-logs) to obtain geometric means and geometric mean ratios. The 90% confidence intervals for geometric means were calculated for AVI-005 5 MIU and control interferon groups.

At the 5 MIU dose level, an unequal variances t-test (with Satterthwaite degrees-of-freedom) was used to compare AVI-005 and control interferon for parameters $AUC_{0\text{-}24\ hr}$ and C_{max}. Ratios (AVI-005/control interferon) of geometric means were calculated by back-transforming the difference of means, and a 90% confidence interval was calculated for this ratio. Residuals were computed and examined for normality. Summary statistics (minimum, maximum, standard deviation, and median or harmonic mean) were computed for half-life (harmonic mean) and T_{max} (median) values.

Pharmacodynamics

Pharmacodynamic analysis consisted of serial determination of serum analytes neopterin and β2 microglobulin and mRNA expression of PKR and OAS. These were analyzed as fold increase from the baseline for each, with determination of an area under the fold increase curve and C_{max}, the maximum increase from baseline. Geometric mean ratios of $AUC_{0\text{-}144\ hr}$ and C_{max} were compared, with 90% confidence intervals calculated for AVI-005 5 MIU and control interferon groups. Measurement of neopterin and β2 microglobulin was performed at predose, 24, 48, 72, 96, 120, and 144 hours post dose. One half of the subjects in the control interferon group had these analytes measured only at predose, 24, 48, and 144 hours. One subject was not included in the analysis of neopterin and β2 microglobulin due to a very high baseline neopterin. This subject did not have any evidence of viral infection at the time of dosing, but did develop some symptoms of upper respiratory tract infection in the subsequent 30 days, which may account for this finding.

Data points for neopterin and β2 microglobulin for the 120 hour time point were not available for one subject, and data for the 144 hour time point were not available for 4 subjects. For this reason, both $AUC_{0\text{-}120\ hr}$ and $AUC_{0\text{-}144\ hr}$ were determined. Examination of the geometric mean ratio between AVI-005 5 MIU and control interferon was performed on $AUC_{0\text{-}144\ hr}$ and C_{max}. The $AUC_{0\text{-}120\ hr}$ was used to compare different AVI-005 dose levels.

PKR and OAS mRNA samples were assayed at predose, 8, 12, 24, 48, and 72 hours. Five subjects who received control interferon did not have a 72 hour sample for PKR or OAS. Examination of the geometric mean ratio between AVI-005 5 MIU and control interferon was performed on $AUC_{0\text{-}72\ hr}$ and C_{max}.

RESULTS

Pharmacokinetics

Table 1 shows the PK parameters of the 3 measured groups. Half life, volume of distribution and clearance for the AVI-005 1.66 MIU were not presented because these parameters could be estimated for only one subject of four receiving this treatment. For groups receiving 5 MIU of AVI-005 or control interferon, these parameters were generated only in whom reliable terminal elimination phases could be determined. Interferon concentrations could not be reliably quantified in one subject receiving control interferon. This subject did, however, mount pharmacodynamic responses suggestive of interferon effect in all 4 analytes measured.

A comparison of the pharmacokinetic parameters of AVI-005 and control interferon at the 5 MIU dose is presented in Table 2. $AUC_{0\text{-}24\ hr}$ and C_{max} for the AVI-005 group was approximately 2 fold greater than in the control interferon group. It should be noted that both AVI-005 and control interferon were dosed on an activity (MIU) basis, though analytic characterization was performed on mass of interferon. To correct for the

differences in mass of interferon administered, individual subject parameters of $AUC_{0\text{-}24\ hr}$ and C_{max} were divided by the mass of drug administered. Normalization of the $AUC_{0\text{-}24\ hr}$ and C_{max} data to mass of drug administered decreased the difference between the two treatments, although $AUC_{0\text{-}24\ hr}$ of AVI-005 remained significantly larger than that of control interferon, ($p=<0.001$). The 90% confidence intervals for both dose-normalized $AUC_{0\text{-}24\ hr}$ and C_{max} extended higher than the customary upper limit pharmacokinetic geometric mean ratio bound of 1.25 for bioequivalence.

Pharmacodynamics

Neopterin AUC and C_{max} rose with increasing administered dose of AVI-005 (Figure 1). β2 microglobulin demonstrated a similar pattern of dose-dependent increase in AUC and C_{max} (Figure 2). At the 5 MIU dose, control interferon and AVI-005 had comparable neopterin and β2 microglobulin $AUC_{0\text{-}144\ hr}$ and C_{max} (Table 3). The only statistically significant finding was an increase of control interferon β2 microglobulin in $C_{max.}$ For all treatment groups, there was an increase in OAS and PKR over baseline values (Figures 3 and 4). However, there was no appreciable dose relationship of AVI-005 to induction of either OAS or PKR. Control drug generated a larger OAS $AUC_{0\text{-}72\ hr}$ and C_{max} compared to AVI-005 5 MIU, though the confidence intervals around the point estimate of the geometric mean ratios was large.(Table 4) Differences in PKR between each of these treatments was less, but there remained a fair amount of intersubject variability response.

Safety Results

Twenty seven of the 28 subjects reported at least 1 adverse event. Threee subjects in the AVI-005 0.5 MIU group reported adverse events. Four subjects in the AVI-005 1.66 MIU group reported adverse events. Ten subjects in the AVI-005 5 MIU, as well as 10 subjects in the control interferon group reported adverse events. A summary of adverse events is presented in Table 5..

DISCUSSION

This study describes the first human experience with AVI-005, an avian derived, human IFN-α2b. Unlike the presently commercially available IFN-α2b products, Intron A (control interferon) and PEG Intron, AVI 005 has glycosylation that endows the drug with a structure very similar to the endogenously produced IFN-α2b. In this rising single dose phase 1 study, AVI-005 demonstrated a safety profile that was similar to that of an equivalent unit dose of control interferon, with no unanticipated toxicities observed.

Biological markers were measured to assess the in vivo pharmacologic response to AVI-005. Though a direct dose:response relationship has not always been reported, there is consensus that the markers used in this study demonstrate biological response to interferons. (3, 13-17). There was an increase in the pharmacodynamic response markers of neopterin and β2 microglobulin to increasing doses of AVI-005, though the small number of subjects in the lower dose ranges and sequential dose escalation design precluded a formal assessment of dose response linearity. Though AVI-005 clearly induced OAS and PKR mRNA translation, there did not appear to be as clear a relationship in magnitude of change from baseline to dose administered. It should also be noted that the present study differs from others in the use of mRNA expression of OAS and PKR, rather than actual measurement of these proteins, though it can be inferred that the two should be linked. In addition to measured pharmacodynamic responses, there was also a dose-related increase in adverse events, which also supports the biologic activity of AVI-005. In contrast to the dose dependency of most of the pharmacodynamic responses, there was a lack of apparent pharmacokinetic dose response. This is likely a function of small number of subjects in the AVI-005 1.66 MIU group, drug concentrations near the lower limit of quantification in this group and a large degree of intersubject variability.

While there were no *a priori* equivalence or superiority hypotheses tested, pharmacokinetic and pharmacodynamic comparisons between AVI-005 and control interferon can be made. The T_{max} was similar for

both drugs, suggesting a similar rate of distribution or uptake from site of intramuscular injection, though formal comparisons of relative bioavailability could not be determined since administration of drug by the intravenous route was not performed. At a comparable dose of control interferon on an activity basis, AVI 005 demonstrates greater systemic exposure to mass of drug, as measured $AUC_{0\text{-}24\ hr}$. This difference was decreased, but remained to some degree, after normalization for mass of drug administered. AVI-005 and control interferon can not be considered bioequivalent by strict pharmacokinetic standards, as the 90% confidence intervals of the geometric mean ratios of the two drugs for $AUC_{0\text{-}24\ hr}$ and C_{max} lie outside of the generally accepted 0.8-1.25 bounds and the study was not conducted in a crossover design in the same individual. Direct statistical comparisons of half life, volume of distribution and clearance between the two treatments are not appropriate due to the inability to measure a terminal elimination phase in a number of individuals, as described earlier. However, it can be hypothesized that the more glycosylated conjugation of AVI-005 probably does not confer the increase in half life bestowed by polyethylene glycol conjugation, as seen with PEG-Intron.

There were no protocol-specified *a priori* pharmacodynamic bounds to compare AVI-005 and control interferon. However, using bioequivalence bounds of 0.5-2.0 for the geometric mean ratio between the two drugs, there did appear to be a comparable response in the markers of neopterin and β2 microglobulin. For the pharmacodynamic markers of OAS transcription, control interferon generated a larger response than AVI-005, while the opposite was true for PKR. However, it should be noted that the intersubject variability for OAS and PKR were large, making any firm conclusion about the relative potency of each drug on these markers difficult. In addition, the sensitivity of these markers to discriminate relative biologic potency, nor the validity of these markers as surrogate endpoints for clinical efficacy, have not been definitively established

In conclusion, a similar dose of the avian- produced human IFN-α2b AVI-005 generates a larger $AUC_{0\text{-}24hr}$ than the control interferon Intron A, a commercially available recombinant interferon -α2b with years of clinical experience and proven efficacy. AVI-005 has a safety profile with a single dose that appears to be comparable to that of control interferon, and generates a similar pharmacodynamic response when administered in an equipotent dose. It is not known if the glycosylation of AVI-005 provides any advantages compared to non-glycosylated versions with multiple doses in patients with interferon-remediable disease. Given the potential reduced cost of production of this product compared to standard interferons, AVI-005 may expand the use of IFN-α2b to populations in the world whom at the present time do not have access to this product.

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Conflicts of Interest

The authors who have taken part in the research of this paper have (Parker) and have not (Patel, Pequignot, Greenberg, Kraft) a relationship with the manufacturers of the drug involved either in the past or present. Please note that Drs. Parker was an employees of AviGenics, Inc. at the time the study was conducted. Dr. Leavitt is an employee of AviGenics, Inc. Drs. Patel, Greenberg, Kraft and Mr. Pequignot have not had a relationship with AviGenics outside of receiving support to conduct this study.

Table 1. Summary of Mean Values of Each of the Treatment Groups

		AVI-005 1.66 MIU		AVI-005 5 MIU		Control Interferon 5 MIU	
Parameter	**Unit**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Half life	hr	ND[d]	ND[e]	7.1 [b]	2.0	6.1 [c]	2.4
T_{max}	hr	10[a]	NA	5[a]	NA	7[a]	NA
C_{max}	pg/mL	239	124	237	93.5	122	52
$AUC_{0\text{-}24\ hr}$	hr*pg/mL	3000	1810	2720	1250	1090	321
Volume of Distribution	L	ND[d]	ND[e]	114 [b]	43	154 [c]	55
Clearance	L/hr	ND[d]	ND[e]	12 [b]	6	18 [c]	5

[a] Median
[b] Determined from 6 individuals
[c] Determined from 5 individuals
[d] Half life, Volume of Distribution and Clearance were not presented because these parameters could be estimated for only one subject of four receiving this treatment
[e] Missing data
NA= non-applicable
ND= not done

Table 2. Geometric Mean Ratios of AVI-005 5 MIU and Control Interferon and Geometric Mean Ratios Normalized to Mass of Drug Administered ($AUC_{0\text{-}24\ hr}$ and C_{max})

		Geometric Means		Ratio of Geometric Means (AVI-005/ Control Interferon) and 90% Confidence Interval
Parameter	Units	AVI-005 (N = 10)	Control interferon (N = 9)	
$AUC_{0\text{-}24\ hr}$	hr•pg/mL	2,510	1,050	2.37 (1.80, 3.13)
C_{max}	pg/mL	220	114	1.94 (1.42, 2.66)
T_{max} †	hr	5.00	7.00	
$AUC_{0\text{-}24\ hr}$ dM	hr•pg/mL/ug	80	55	1.46 (1.10, 1.93)
C_{max}dM	pg/mL/ug	7	6	1.19 (0.87, 1.63)

† Median

dM = normalized to mass of drug administered

Table 3. Fold Increase in β2 Microglobulin Following AVI-005 5 MIU or Control Interferon

Parameter (units)	Pharmacodynamic Marker	Geometric Means		Ratio of Geometric Means (AVI-005/ Control Interferon) and 90% Confidence Interval
		AVI-005 (N = 10)	Control Interferon (N = 5)	
$AUC_{0\text{-}144\ hr}$ (fold increase-hr)	β2 microglobulin	180	200	0.92 (0.84, 1.00)
C_{max} (fold increase)	β2 microglobulin	1.6	2.0	0.81 (0.71, 0.91)
$AUC_{0\text{-}144\ hr}$ (fold increase-hr)	Neopterin	370	402	0.92 (0.67, 1.27)
C_{max} (fold increase)	Neopterin	4	5	0.87 (0.65, 1.18)

Table 4. Fold Induction for PKR and OAS Following AVI-005 5 MIU or Control Interferon

	Parameter (units)	Geometric Means		Ratio of Geometric Means (AVI-005/ Control Interferon) and 90% Confidence Interval
		AVI-005 (N = 10)	Control Interferon (N = 5)	
OAS	$AUC_{0\text{-}72\ hr}$ (fold increase-hr)	570	866	0.66 (0.32, 1.35)
	C_{max} (fold increase)	25	34	0.73 (0.34, 1.58)
PKR	$AUC_{0\text{-}72\ hr}$ (fold increase-hr)	397	344	1.15 (0.68, 1.96)
	C_{max} (fold increase)	21	16	1.27 (0.67, 2.44)

Table 5. Number of Subjects with Most Common Adverse Events

	AVI-005 0.5 MIU N = 4	AVI-005 1.66 MIU N = 4	AVI-005 5.0 MIU N = 10	Control Interferon 5.0 MIU N = 10
Total Subjects with Adverse Events[a]	3	4	10	10
Adverse Event [b]				
Pyrexia	0	0	9	10
Headache	2	3	8	8
Chills	0	0	7	8
Myalgia	0	1	3	2
Nausea	0	1	2	0
Pharyngolaryngeal pain	1	0	2	0
Pain in extremity	0	1	1	1
Back pain	0	1	0	2
Average Number of Adverse Events per Subject[c]	0.75	1.75	3.20	3.10

[a] Includes subjects experiencing at least one of the most common adverse events (>10% of all subjects).
[b] Multiple occurrences of the same adverse event within a subject are counted once.
[c] Average is generated by dividing the total number of adverse events in a group by the number of subjects in the respective group.

Figure 1: Neopterin (% change from baseline)

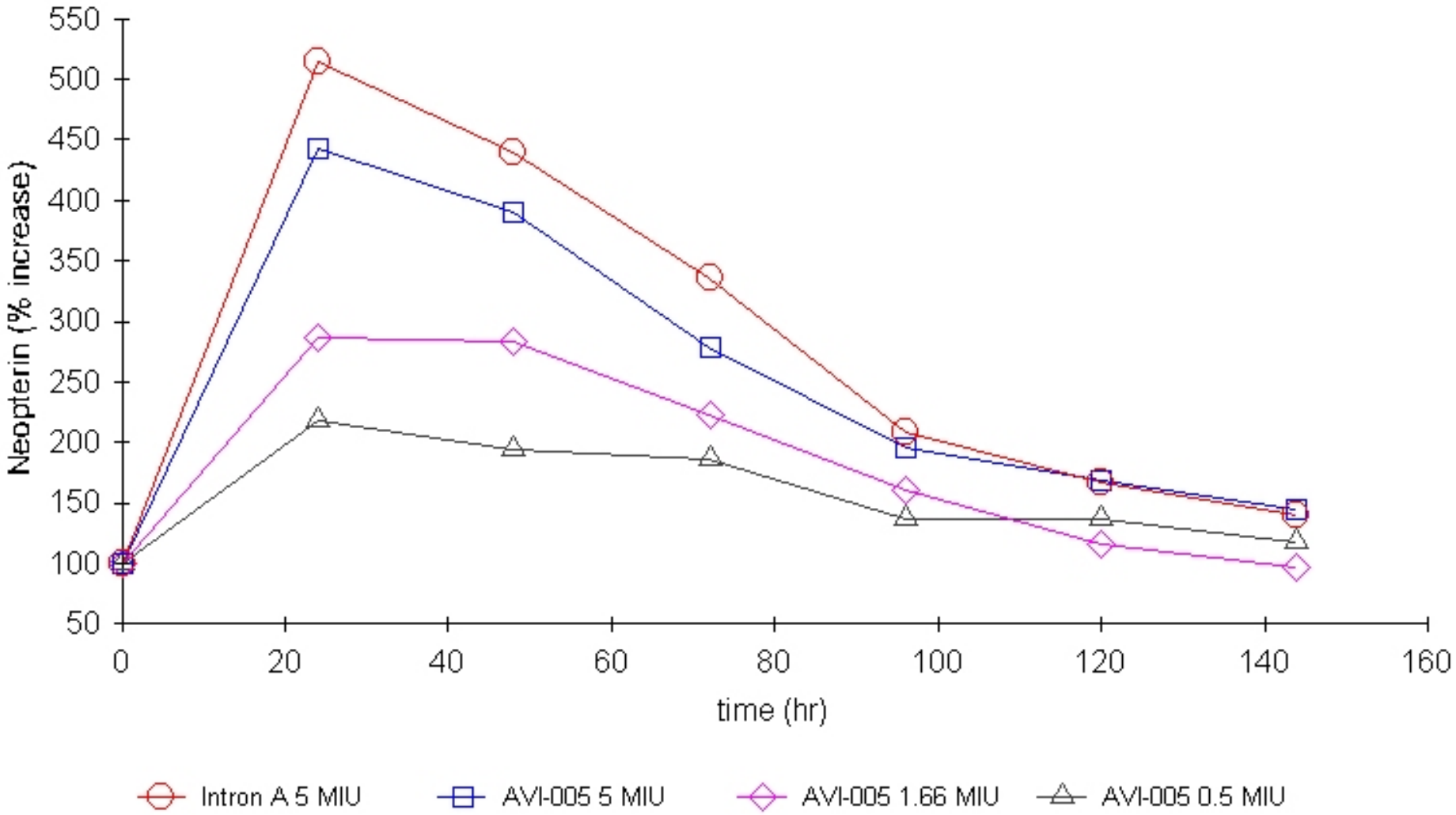


Figure 2: β2 Microglobulin (% change from baseline)

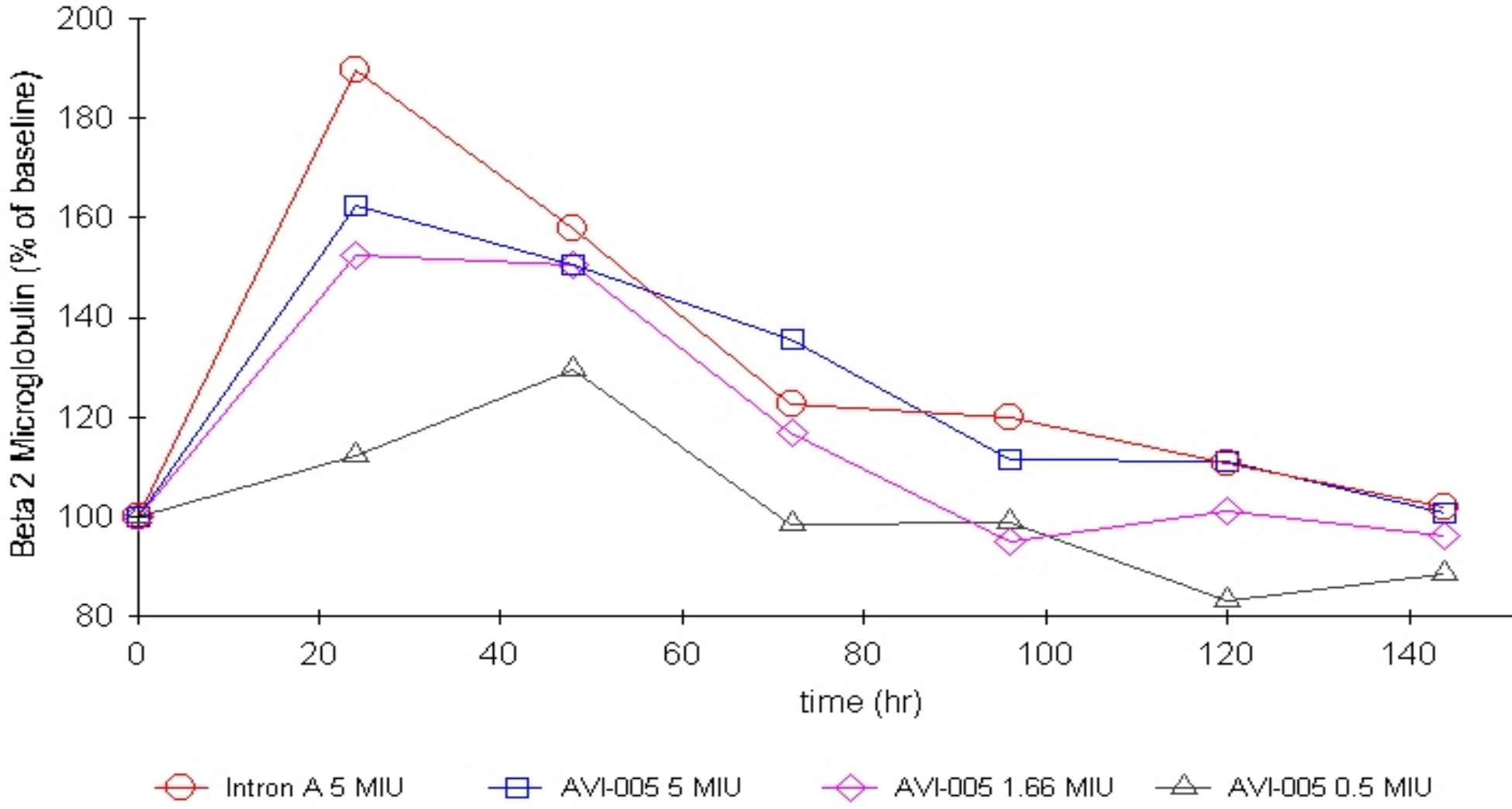


Figure 3: 2'5' oligoadenylate synthetase (% change from baseline)

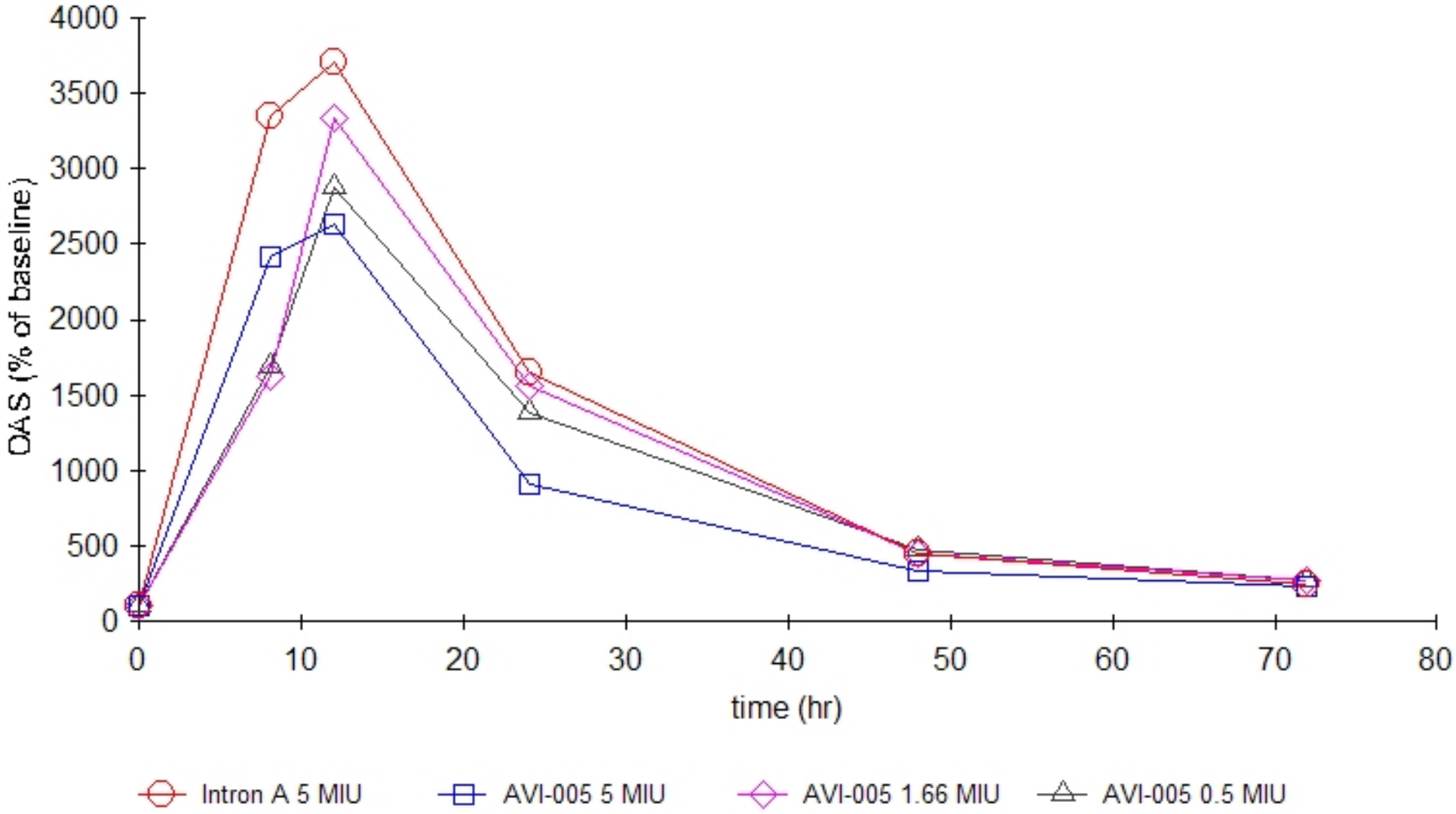
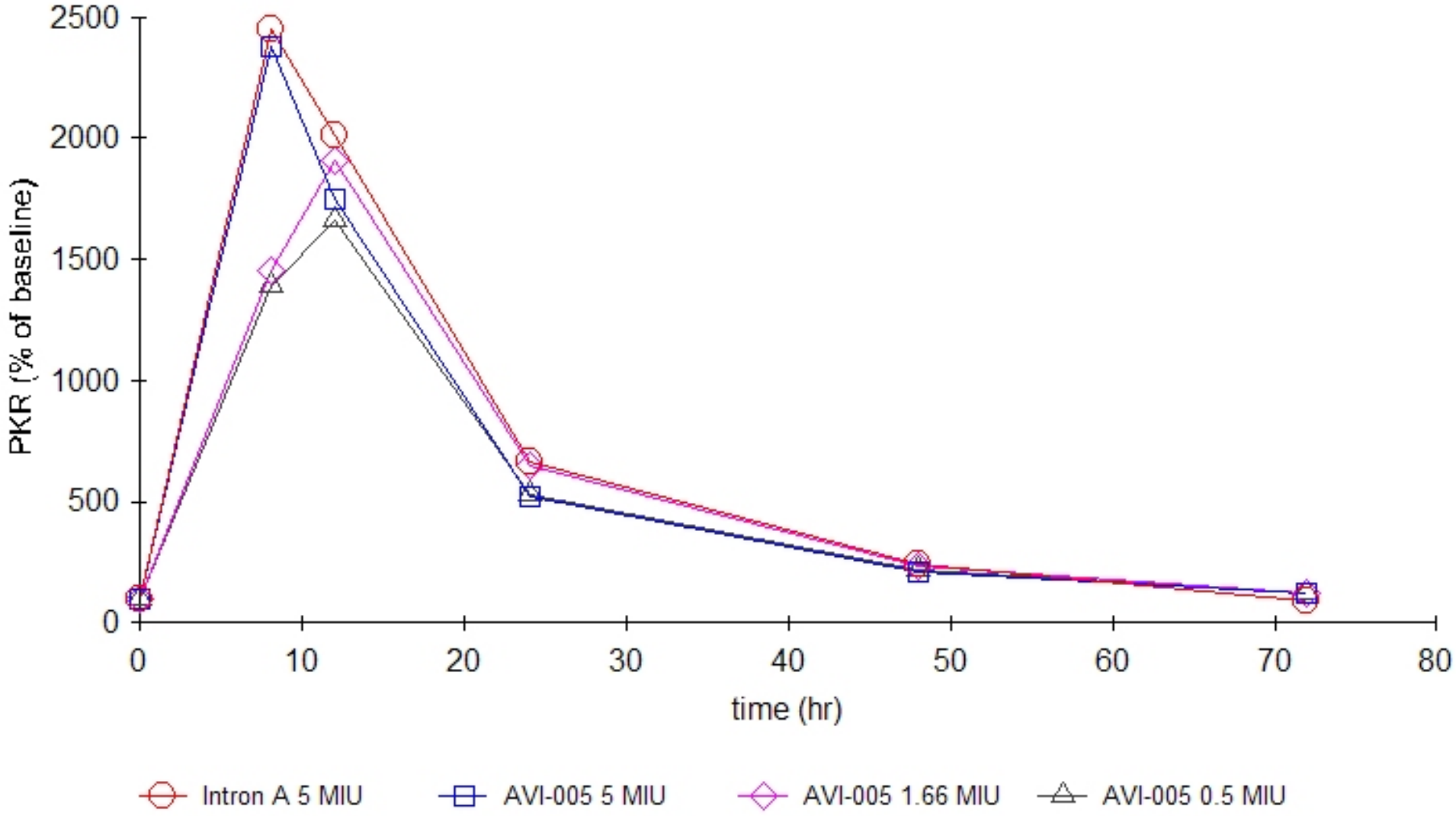


Figure 4: Interferon-inducible protein kinase (% change from baseline)



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