Evaluation of Immunotherapeutic Effects of *Aloe vera* Polysaccharides Against Coccidiosis in Chicken

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Abstract

This study reports the immunotherapeutic effects of *Aloe (A.) vera* polysaccharides against coccidiosis in broiler chicken. For the purpose, polysaccharides were recovered from *A. vera* and analyzed by using HPLC. Three different hexose sugars including maltose, glucose and mannose were detected in hydrolyzed solution of *A. vera*. The extracted polysaccharides (graded doses) were evaluated for immunotherapeutic activities against coccidiosis in chicken. Results revealed that percent protection and daily weight gains were significantly higher (P<0.05) in chicken administered with *A. vera* polysaccharides as compared to control group. On the other hand, oocyst counts and lesion scores were lower (P<0.05) in polysaccharides administered chickens as compared to control. Moreover, anti-coccidial indices were also higher in chickens administered with polysaccharides (159.75-239.63) as compared to control (36.57). Except spleen, the organ-body weight ratios of all lymphoid organs of experimental and control groups were statistically similar (P>0.05). Based upon findings of this study, it was concluded that *A. vera* derived polysaccharides had immunotherapeutic activity against coccidiosis in chickens and might be further explored for its commercial feasibility for effective use in poultry industry to control avian coccidiosis.

Keywords: Immunotherapeutic, Aloe vera, Polysaccharides, Coccidiosis, Chicken

INTRODUCTION

Coccidiosis is one of the most important protozoal infections of poultry industry, inflicting heavy economic losses in the form of high morbidity and mortality in affected flocks [1]. It is caused by different species of genus *Eimeria*, belonging to family *Eimeriidae*. Poor management such as damp litter, contaminated drinkers and feeders, high stock density and poor ventilation are the most important predisposing factors of this disease in intensive poultry production [1]. It has a negative impact on the production performance of affected birds in terms

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of retarded growth and poor feed conversion ratios in
addition to high morbidity and mortality [3]. According
to an estimate, it causes economic losses up to three billion
US dollars annually worldwide [4,5].

In current era, the poultry industry largely relies upon
the use of chemophylactic drugs and live vaccines
to control coccidiosis [6,7]. At the same time, there is also
increasing concern towards the use of alternative control
measures due to some associated pitfalls of exiting control
strategies including the emergence of drug resistant
strains, high cost of vaccines and drug residues in meat
and eggs [5,8]. At present, many alternative strategies are
under investigation for effective, economical and environ-
ment friendly control of coccidiosis, including the use
of medicinal plants [9,10]. In this context, A. vera has been
reported for promising immunomodulatory effects in
different animal models, highlighting it as a potential
candidate for immunotherapy in different ailments [11].

A. vera is one of the most commonly used medicinal
plants throughout the world with pronounced historical
importance [12]. Its gel contains more than 75 active
components including polysaccharides, minerals, phenolic
compounds, proteins, sugars, vitamins, amino acids and
saponins each with some pharmacological effects in
different ailments [13,14]. Literature revealed that most of
medicinal effects are due to polysaccharides present
in inner leaf gel [15]. These polysaccharides had been
extensively reported as a wound healing agent in different
wound conditions by proliferation of fibroblasts and
hydroxyproline and hyaluronic acid production in fibro-
blasts and thus extracellular remodeling in wound healing
process [16]. It could inhibit inflammatory process by
reduction of leukocytes adhesions and pro-inflammatory
cytokines [17]. Its administration increases phagocytic
and proliferative activity of reticuloendothelial system [18].
The A. vera polysaccharides are well documented for
pharmacological activities in different animal disease
models but only a few studies are available in the
chicken model. Keeping in view, in continuation to our
previous studies [12,19], this study aimed to investigate the
immunotherapeutic efficacy of A. vera polysaccharides
against coccidial infection in chickens.

**MATERIAL and METHODS**

**Procurement and Processing of A. vera Leaves**

Fresh leaves of A. vera were obtained from Botanical Garden,
University of Agriculture, Faisalabad (UAF), Pakistan and
their authenticity was confirmed from the concerned
botanist of Department of Botany, UAF, Pakistan. The plant
specimens were kept in the Ethnoveterinary Research
and Development Centre, Department of Parasitology,
UAF, Pakistan (Specimen Voucher No. 072). Fresh leaves
after harvesting were subjected to surface sterilization by
washing with chlorinated H2O followed by formalin (0.005
ppm solution) and finally with distilled H2O [19].

**Separation of Leaf Gel**

The mucilaginous leaf gel was separated from A. vera leaves
within 3-4 h post collection to avoid aerodeterioration
of gel contents. Briefly, the prewashed A. vera leaves were
incised longitudinally with the help of a sharp sterilized
knife followed by gentle scraping of gel using a spatula.
The gel was homogenized, filtered through cheese cloth and
stored in screw capped jars at 4°C till further use.

**Extraction and Hydrolysis of A. vera Polysaccharides**

Polysaccharides were extracted from A. vera gel by
following the methodology described by Chang et al. [20]
with minor modifications. In brief, the A. vera gel was
mixed with 95% ethanol (1:4) by vigorous shaking and
incubated for 12 h at 4°C. The supernatant was discarded,
and precipitate was subjected to centrifugation (6500×g
for 10 min). The precipitate was mixed with dd-H2O and
incubated for 12 h and again precipitated with 95% ethanol
(1:4). The procedure was repeated several times until all
the colored material was removed. The final precipitate
was mixed with dd-H2O and treated with Sevag reagent
[butanol:chloroform (1:4 v/v)]. The protein contents were
removed by repeated oscillation and centrifugation procedures [21]. The deproteinated solution was mixed
with 95% ethanol (1:3) to precipitate the polysaccharides.
The precipitated polysaccharides were separated and
subjected to further purification by washing 2-3 times with
ethanol (absolute) followed by acetone and ethyl ether,
respectively. Polysaccharides thus obtained were dried at
40°C for 24-48 h.

**HPLC Analysis of Polysaccharides**

The extracted polysaccharides were hydrolyzed to get the
monomer units (monosaccharides) as described previously [22]
with minor modifications. In brief, the polysaccharides
were refluxed in trifloroacetic acid (2M; Sigma-Aldrich®,
USA) at 100°C for 2 h in a round-bottom flask equipped
with a reflux condenser. The TFA and water contents
were removed by evaporation (−65°C), respectively. Polysaccharides thus obtained were analysed by Shimadzu-10A
HPLC workstation (Japan) equipped with a quaternary gradient pump unit and a refractive index detector (RID). The Rezex RCM-
Monosaccharide Ca²⁺ column (Phenomenex, USA) was
used to get absorption spectra at a wavelength of 235
nm at 80°C. Isocratic DD H2O was used as mobile phase.
Injection volume for each of monosaccharide standards
and sample was taken as 20 μL.

**The Infective Material**

Sporulated oocysts of mixed Eimeria (E.) species including
E. tenella, E. acervulina, E. maxima and E. necatrix maintained
at Immunoparasitology Laboratory, UAF, Pakistan were
used for this study to induce Eimeria infection in the
birds. The infective dose was adjusted at the rate of 7×10⁴
sporulated oocysts of per 2 mL of phosphate buffered saline (PBS).

**Experimental Design**

A total of 160 (one-day-old) broiler chicks were obtained from local market and reared under standard management conditions at Experimental Poultry Shed, Department of Parasitology, UAF. All the chicks were offered withdrawal feed and water *ad libitum* throughout the study and vaccinated according to the routine schedule against ND, IBD and HPS. During the experimental trial, all procedures were performed in accordance to the guidelines of the Institutional Animal Care and Use Committee of UAF.

After 5 days of acclimatization, chicks were randomly divided into four equal groups A1-A4, each containing 40 chicks and were administered orally with graded doses of Aloe polysaccharides for three consecutive days i.e. 5th–7th days of age. Groups A1, A2 and A3 were administered A. vera polysaccharides at the dose rates of 100, 200 and 300 mg.kg⁻¹ body weight, respectively while group A4 was kept on PBS as a control.

**Immunotherapeutic Evaluation**

On day 14th post-administration of A. vera polysaccharides, chickens of all the groups were challenged with infective dose of mixed species of genus *Eimeria* (local isolates; 7×10⁴ sporulated oocysts per bird) with the help of an oral gavage. In each group, chicks were monitored for oocysts per gram of faeces (OPG), daily weight gains, lesion scoring and mortality from day 3rd to 12th post challenge. For lesion scoring, dead and survived chickens in all the groups were killed humanely and scored for intensity of lesions as described by Johnson and Reid.

Further, the percent protection against lesions was calculated by using the formula described by Singh and Gill as follows:

\[
\text{Percent protection against lesions} = \left( \frac{\text{Average lesion score (IUG)} - \text{Average lesion score (IMG)}}{\text{Average lesion score (IUG)}} \right) \times 100
\]

Where,

- IUG = Infected Untreated Group; IMG = Infected Medicated Group

**Anti-coccidial Index (ACI)**

Anti-coccidial index (ACI) was calculated to demonstrate the therapeutic efficacy of A. vera polysaccharides by following the formula described by Shah et al. as follows:

\[
\text{Relative rate of weight gain} = \frac{\text{Relative weight gain in control group}}{\text{Relative weight gain in A. vera groups}}
\]

Relative rate of weight gain was calculated by subtracting the body weight at the time of challenge from the body weight at the end of experiment. Survival rate was estimated by the number of survived chickens divided by the initial number of chickens. Lesion scores of the chickens from all groups were calculated by the method of Johnson and Reid and oocyst value was calculated by using the formula described previously as follows:

**Development of Lymphoid Organs**

The organ-body weight ratio of lymphoid organs including spleen, thymus, caecal tonsils and bursa of Fabricius were calculated on day 12th post challenge with *Eimeria* species. Briefly, chickens of all the groups were weighed individually. Thereafter, birds were killed humanely and their lymphoid organs were incised out and weighed. The results were expressed as percent organ-body weight ratios as described earlier.

**Statistical Analysis**

Data thus collected were analyzed by using statistical analysis software (SAS® 2004) through one-way ANOVA and Duncan’s Multiple Range (DMR) test. The differences were considered significant at P<0.05.

**RESULTS**

**HPLC Profile of A. vera Polysaccharides**

HPLC analysis of the hydrolysed solution of A. vera polysaccharides revealed the presence of three different monosaccharide units including mannose, glucose and maltose at peak retention times (min) of 12.55, 11.08 and 9.423, respectively. Molar concentrations (%) of detected monosaccharides are presented in Table 1.

**Immunotherapeutic Evaluation of A. vera Polysaccharides**

- **Oocyst Counts and Daily Weight Gains Post-Challenge:**
  All the groups administered with graded doses of A. vera polysaccharides showed significantly lower (P<0.05) oocyst counts as compared to control from days 4th to 12th post challenge. Maximum OPG was recorded on day 9th post infection in all groups. OPG count was lower in group A2 as compared to A1 and control. However, the

<p>| Table 1. Quantitative analysis of monosaccharides detected in the hydrolyzed solution of A. vera polysaccharides |
|-------------------------------------------------|--------|---------|--------|----------|</p>
<table>
<thead>
<tr>
<th>Monosaccharides</th>
<th>Retention Time (min)</th>
<th>Area (mV.s)</th>
<th>Height (mV)</th>
<th>Quantity (molar %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltose</td>
<td>9.423</td>
<td>11.837</td>
<td>0.54</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose</td>
<td>11.08</td>
<td>35.252</td>
<td>0.605</td>
<td>0.11</td>
</tr>
<tr>
<td>Mannose</td>
<td>12.55</td>
<td>36.885</td>
<td>0.612</td>
<td>0.02</td>
</tr>
</tbody>
</table>
difference between groups A₂ and A₃ was statistically non-significant (P>0.05; Fig. 1). On the other hand, daily weight gains were significantly higher (P<0.05) in chickens administered with different doses of A. vera polysaccharides as compared to those of control group; although no graded dose response was detected (Fig. 2).

- Percent Protection, Lesion Scoring and Percent Protection Against Lesions: The highest protection (70%) was observed in group A₁ administered with A. vera polysaccharides (200 mg.kg⁻¹ BW) followed by group A₃ (60%) and group A₂ (55%) and control group (30%). Chickens of all the groups (both survived and dead chickens) were examined for lesion scoring on a scale from 0 to 4. Chickens of experimental groups administered with A. vera polysaccharides showed lesser lesions and thus higher percent protection against lesions as compared to those of control group. Among experimental groups, chickens of group A₂ showed the lowest score of severe lesions followed by A₁ and A₃ as compared to chickens in control group, which showed severe lesion scores (Table 2).

- Estimation of Anti-coccidial Index: The group A₂ administered with A. vera polysaccharides at the rate of 200 mg.kg⁻¹ showed the highest anti-coccidial index (239.63) followed by those of groups A₃ (195.31) and A₁ (159.75). A value of 36.57 was also recorded for control group that could be due to the self-limiting nature of the Eimeria infection in poultry (Table 3).

Organ-body Weight Ratios in A. vera Polysaccharides Administered and Control Groups: All with mixed Eimeria groups showed statistically similar organ-body weight ratios (P>0.05) except spleen, which showed significantly higher (P<0.05) spleen-body ratio in birds of control group as compared to those administered with A. vera polysaccharides (Table 4).

Table 2. Lesion scoring and percent protection against lesions

<table>
<thead>
<tr>
<th>Group</th>
<th>Lesion Scoring of Birds</th>
<th>Protection Against Lesions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 1 2 3 4</td>
<td></td>
</tr>
<tr>
<td>Caeca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₁</td>
<td>0 5 6 11 18</td>
<td>23.75ab</td>
</tr>
<tr>
<td>A₂</td>
<td>0 8 14 6 12</td>
<td>36.25a</td>
</tr>
<tr>
<td>A₃</td>
<td>0 0 8 16 16</td>
<td>20b</td>
</tr>
<tr>
<td>A₄</td>
<td>0 0 5 11 28</td>
<td>3.25c</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A₁</td>
<td>0 11 6 8 15</td>
<td>33.125b</td>
</tr>
<tr>
<td>A₂</td>
<td>0 12 9 5 9</td>
<td>49.375a</td>
</tr>
<tr>
<td>A₃</td>
<td>0 16 7 5 12</td>
<td>41.875a</td>
</tr>
<tr>
<td>A₄</td>
<td>0 0 2 16 22</td>
<td>12.5c</td>
</tr>
</tbody>
</table>

Values sharing similar letters in a column are statistically non-significant (P>0.05); A₁ = A. vera polysaccharides at dose rate of 100 mg.kg⁻¹ BW; A₂ = A. vera polysaccharides at dose rate of 200 mg.kg⁻¹ BW; A₃ = A. vera polysaccharides at dose rate of 300 mg.kg⁻¹ BW; A₄ = Control group.

Fig 1. Oocysts per gram of faeces from day 4th to 12th post-challenge with Eimeria species. Bars sharing different letters on a particular day present a significant difference (P<0.05). A₁ = A. vera polysaccharides given at rate of 100 mg.kg⁻¹ BW; A₂ = A. vera polysaccharides given at rate of 200 mg.kg⁻¹ BW; A₃ = A. vera polysaccharides given at rate of 300 mg.kg⁻¹ BW; A₄ = Control group.

Fig 2. Daily weight gains of experimental and control chickens from day 3rd to 12th post-challenge. Bars sharing different letters on a particular day present a significant difference (P<0.05). A₁ = A. vera polysaccharides given at rate of 100 mg.kg⁻¹ BW; A₂ = A. vera polysaccharides given at rate of 200 mg.kg⁻¹ BW; A₃ = A. vera polysaccharides given at rate of 300 mg.kg⁻¹ BW; A₄ = Control group.
A. vera plant [15,32]. been isolated from different parts of galactomannan, glucomannan and glucuronic acid had galactogalacturan, galactoglucoarabinomannan, glucocacemannan, arabinoxylan, arabinorhamnogalactan, galactan, mannose. Previously, several polysaccharides including different monosaccharides including maltose, glucose and by using HPLC. Results indicated the presence of three Coccidiosis is an important protozoal infection of poultry DISCUSSION Keeping in view, this study stimulation of phagocytes and humoral immunity in immunostimulatory activities, mainly antioxidant effects; the production performance and thus farm profitability [4,28,29]. of high economic importance having a negative impact on disease is controlled through medication and vaccination strategies but each with certain limitations. As an alternative approach, modern trends are molding towards the use of native biomolecules from different medicinal plants for the treatment of various ailments in both animals and human beings [10]. In this regard, A. vera reported to have significant immunoregulatory and immunostimulatory activities, mainly antioxidant effects; stimulation of phagocytes and humoral immunity in different animal models [12,37]. Yim et al. [38] also reported that A. vera extracts administered at different dose rates had also reported that carbohydrate polymers (glucomannans) inhibited cyclooxygenase pathway resulting in decreased putrefaction that subsided/decreased inflammation [36] or lining of intestine layer with Aloe biomolecules [43]. Improved intestinal health in A. vera polysaccharides administered chickens might be responsible for better higher weight gains and thus better production performance [44].

Maximum protection (70%) in polysaccharides administered chickens might be correlated immunostimulatory activity of Aloe polysaccharides like acemannan which had been reported to reduce the opportunistic infections and stimulate wound healing [45], Further, previous studies reported that carbohydrate polymers (glucomannans) present in A. vera played role in healing process [46] and inhibited cyclooxygenase pathway resulting in decreased prostaglandin production from arachidonic acids [47]. Vahedi et al. [48] reported that A. vera polysaccharides led to stimulate cellular and humoral immune responses by increased synthesis and release of T-lymphocytes and cytokines, which might be speculated to neutralize the pathogenic organisms like Eimeria species. Earlier, A. vera extracts administered at different dose rates had also revealed significantly elevated macrophages and white blood cell counts in mice [49]. Further, Cheesbrough [50] also reported A. vera polysaccharides to boost the activity of intestinal macrophages and T-lymphocytes up to 50 percent to prevent the penetration of pathogenic viruses, and this variability might be associated with isolation methodology along with seasonal and cultivational variations [34].

For immunotherapeutic evaluation of A. vera polysaccharides, chickens of all the groups (experimental and control) were challenged with mixed Eimeria species in this study. Significantly lower oocyst counts in A. vera treated groups might be correlated to the development of resistance induced by A. vera polysaccharides against Eimeria species [12,37]. Yim et al. [38] also reported that A. vera extract can be used as a safe dietary supplement against coccidiosis. Some other studies had also reported the similar findings in broilers and rabbits [39-41]. Lesion score is the most common method for assessing intestinal condition during coccidiosis [42]. Chickens administered with polysaccharides, showed higher daily weight gains and lesser lesions on the caeca and intestine as compared to control. These lesser intestinal lesions might be due to the effects of A. vera on intestinal tract microflora, reduced bowel putrefaction that subsided/decreased inflammation [36] or lining of intestine layer with Aloe biomolecules [43]. Improved intestinal health in A. vera polysaccharides administered chickens might be responsible for better higher weight gains and thus better production performance [44].

**Table 3. Anti-coccidial indices in experimental and control groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relative Rate of Weight Gains</th>
<th>Survival Rate</th>
<th>Lesion Value</th>
<th>Oocyst Value</th>
<th>Anticoccidial Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>162.567</td>
<td>0.55</td>
<td>2.675</td>
<td>0.696</td>
<td>159.75</td>
</tr>
<tr>
<td>A2</td>
<td>241.696</td>
<td>0.7</td>
<td>2.025</td>
<td>0.746</td>
<td>239.63</td>
</tr>
<tr>
<td>A3</td>
<td>197.756</td>
<td>0.6</td>
<td>2.325</td>
<td>0.717</td>
<td>195.31</td>
</tr>
<tr>
<td>A4</td>
<td>40.14</td>
<td>0.3</td>
<td>3.5</td>
<td>0</td>
<td>36.57</td>
</tr>
</tbody>
</table>

A1 = A. vera polysaccharides given at rate of 100 mg.kg⁻¹ BW; A2 = A. vera polysaccharides given at rate of 200 mg.kg⁻¹ BW; A3 = A. vera polysaccharides given at rate of 300 mg.kg⁻¹ BW; A4 = Control group

**Table 4. Organ-body weight ratio post challenge in experimental and control chickens**

<table>
<thead>
<tr>
<th>Group</th>
<th>Thymus (Mean ± SE)</th>
<th>Spleen (Mean ± SE)</th>
<th>Bursa (Mean ± SE)</th>
<th>Caecal Tonsils (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.37±0.01</td>
<td>0.27±0.02</td>
<td>0.26±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>A2</td>
<td>0.38±0.01</td>
<td>0.28±0.01</td>
<td>0.25±0.01</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>A3</td>
<td>0.37±0.01</td>
<td>0.28±0.01</td>
<td>0.26±0.01</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>A4</td>
<td>0.36±0.01</td>
<td>0.29±0.01</td>
<td>0.25±0.01</td>
<td>0.07±0.01</td>
</tr>
</tbody>
</table>

Means sharing similar letters in a column are statistically non-significant (P>0.05); A1 = A. vera polysaccharides given at rate of 100 mg.kg⁻¹ BW; A2 = A. vera polysaccharides given at rate of 200 mg.kg⁻¹ BW; A3 = A. vera polysaccharides given at rate of 300 mg.kg⁻¹ BW; A4 = Control group

**DISCUSSION**

Coccidiosis is an important protozoal infection of poultry of variable molecular sizes have been isolated from A. vera [35,36] and this variability might be associated with isolation methodology along with seasonal and cultivational variations [34].

Polysaccharides isolated from A. vera were analyzed by using HPLC. Results indicated the presence of three different monosaccharides including maltose, glucose and mannose. Previously, several polysaccharides including acemannan, arabinoxylan, arabinorhamnogalactan, galactan, galactogalacturcan, galactoglucoarabinomannan, glucogalactomannan, glucosamann and glucuronic acid had been isolated from different parts of A. vera plant [11,32]. Further, presence of saccharides including aldopentose, glucose, L-rhamnose and mannose in A. vera polysaccharides were also reported [33,34]. Tan et al. [13] analyzed A. vera polysaccharides by HPLC and reported presence of mannose as a monomeric unit of isolated polysaccharides. In other studies, different polysaccharides of A. vera played role in healing process [46] and this study was conducted to evaluate the immunotherapeutic efficacy of A. vera polysaccharides against coccidiosis in chicken.
bacteria and tumor cells. Results of present study also showed a similar response against coccidiosis in terms of higher survival percentage and reduced oocyst counts. Some previous studies on herbal biomolecules also reported the similar findings.  

Anticoccidial index (ACI) reflects a comprehensive ability of any compound against coccidial infection. ACI values lower than 120 depict that compound/drug has no anti-coccidial activity; whereas values between 120 and 160 are considered partially effective but very effective at value > 160 [31]. In present study, A. vera polysaccharides administered at dose rates 200 and 300 mg.kg⁻¹ of body weight showed ACI values higher than 160, so can be considered as very effective immunotherapeutic regimes against coccidiosis. A. vera polysaccharides did not show any significant effect on the development of different immune organs including thymus, caecal tonsils and bursa of Fabricius as compared to control. Only spleen-body weight ratios of chickens of control group showed significant difference from those administered with A. vera polysaccharides. Contrary to this, Darabighane et al. [45] reported a significantly higher relative weight of spleen in A. vera gel administered chickens; whereas, some previous similar studies reported a non-significant impact of herbal biomolecules on the development of lymphoid organs. In this study, higher spleen-body weight ratio might be speculated due to cellular infiltration and spleen hypertrophy due to severity of coccidial infection in control group as compared to Aloe polysaccharide administered groups [52,33]. In conclusion, A. vera polysaccharides demonstrated the immunotherapeutic efficacy against coccidiosis in chickens and can be used successfully as a trustworthy alternative to anti-coccidial drugs, against which resistance has been emerged, to combat the avian coccidiosis.

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Evaluation of Immunotherapeutic ...


